JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1033-1041

Molecular Characterization of *Escherichia coli* O157:H7 from Retail Beef in Erzurum, Turkey

G. Adiguzel[1], M. Gulluce[2], C. Bozoglu[3], D. Yanmis[2], A. Gormez[4], M. Atasever[1] and A. Adiguzel[3]*

[1]Department of Food Science and Technology, Faculty of Veterinary Science, Atatürk University, 25240, Erzurum, Turkey.
[2]Department of Biology, Faculty of Science, Atatürk University, 25240, Erzurum, Turkey.
[3]Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, 25240, Erzurum, Turkey.
[4]Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey.

(Received: 28 April 2012; accepted: 08 June 2012)

Ground meat is the leading fresh meat product for microbial contamination when considered by physical properties and preparation conditions. This study was conducted to determine the phenotypic and genotypic characterization of *Escherichia coli* O157 strains isolated from one hundred and forty meat samples obtained from retail markets and butchers, Erzurum, Turkey. As a result %5 of meat samples are determined to contain target microorganism. Fatty acid profiles, metabolic fingerprints (BIOLOG), ERIC-PCR, REP-PCR, BOX-PCR and $(GTG)_5$-PCR profiling methods were used for the phenotypic and genotypic characterization of O157:H7 isolates. The data of fatty acid analysis showed the presence of 20 different fatty acids in the 13 bacterial strains examined. All test strains were identified up to *E. coli* species level with Biolog system. After evaluating several primer sets targeting the repetitive DNA elements of REP, ERIC, BOX and $(GTG)_5$, the ERIC and $(GTG)_5$ primers were found to be the most reliable technique for identification and taxonomic characterization of *E. coli* O157:H7 strains. Therefore, rep-PCR fingerprinting using the ERIC and $(GTG)_5$ primers can be considered as a promising genotypic tool for the identification and characterization of *E. coli* from species to serotype level.

Key words: *Escherichia coli* O157:H7, ground meat, FAMEs, BIOLOG and rep-PCR.

Isolation and enumeration of coliforms and especially *E. coli* are very important for determination of food's hygiene. Because, they are microbial contamination marker in food and water and their presence in food and water indicate that these materials are contaminated with other enteric pathogens[1].

* To whom all correspondence should be addressed.
Tel.: +90 442 231 4450; Fax: +90 442 236 0948.
E-mail: adiguzel@atauni.edu.tr, adiguzel25@gmail.com

Enterohemorrhagic *E. coli* (EHEC) categorized as verotoxin-producing *E. coli* (VTEC), also known as Shiga-toxin producing *E. coli* (STEC), is a group of well-recognized pathogens that are responsible for serious diseases such as hemorrhagic colitis, hemolytic-uremic syndrome and thrombotic thrombocytopenic. Serotype O157 producing flagella of H7 serotype (O157:H7) or not producing flagella (O157:H^-), hereinafter shortened as O157:H7/H^-, are important foodborne pathogens. The organisms usually produce one or both of two Shiga toxins (Stxs: *Stx1* and *Stx2*). Natural reservoirs of these organisms are cattle

and other domestic animals. Therefore, beef, dairy products, and related foods are the primary source of *E. coli* O157:H7/H- infection in humans purpura[2-5]. For elimination of STEC and EHEC from bovine gastrointestinal tracts before the butchering would constitute the first barrier needed to prevent the introduction of these bacteria in to the food chain[6].

The researchers have developed many different culture mediums for the isolation of *E. coli* O157:H7 like Sorbitol MacConkey agar (SMAC) used most common for *E. coli* O157:H7 isolation[5]. Sorbitol MacConkey agar or modified forms of this medium, widely used, have been produced by using the property of *E. coli* O157 strains that not fermenting sorbitol, whereas many other serogroups of *E. coli* ferment. Sorbitol-MacConkey has been modified with cefixime and potassium tellurite supplement (CT-SMAC) which allowed the growth of all strains of Verotoxin producing *E. coli* O157 tested, but completely inhibited the growth of 67% of non-verocytotoxigenic *E. coli* O157 and 97% of strains of other genera including *Proteus* spp., *Aeromonas* spp., *Morganella* spp., and *Providencia* spp.[7]. A novel commercial chromogenic medium (CHROMagar O157), on which commensal *E. coli* form blue or rarely colorless colonies, while strains of STEC O157 form characteristic mauve colonies has been reported to be a sensitive and specific medium for EHEC O157 [8]. But, the high cost of this medium would probably preclude their use in the majority of laboratories[7].

Furthermore, other than the culture methods which are based on biochemical characteristics of the bacteria, many methods have been developed, including serological techniques, which uses both polyclonal and monoclonal antibodies specific for the O and H antigens[9,10]. Molecular approaches have also been practiced. Advances in molecular biology techniques such as whole-cell fatty acid analysis, carbon source utilization analysis, carbon pattern utilization, antibiotic resistance analysis, ribotyping, pulse-field gel electrophoresis, repetitive extragenic palindromic-PCR and microarray techniques, and 16S rRNA sequencing have provided the excellent opportunity for identification and characterization purposes of microorganism at species, subspecies, and serotype levels[11-15].

Fatty acid methyl ester (FAMEs) analysis offers a rapid, easy and cheap method for identification of bacteria from various sources. Whole-cell fatty acid (FA) profiles that have become increasingly important in bacterial identification have been used in bacterial classification for over 35 years. The Biolog system was developed by Biolog, Inc. (Hayward, CA, USA) for identification of Gram-negative and -positive bacteria according to their metabolic fingerprinting. This method is based on metabolic fingerprinting by determination of carbon source utilization profiles. This method is not yet accurate enough to serve as a primary method for identifying many bacteria[16].

In addition, rep-PCR is based on the usage of outwardly facing oligonucleotide PCR primers complementary to interspersed repetitive sequences. And, this technique enables the amplification of differently sized DNA fragments lying between these elements. BOX, ERIC (Enterobacterial Repetitive Intergenic Consensus), REP (Repetitive Extragenic Palindromic) and $(GTG)_5$ can be counted as examples of evolutionarily conserved repetitive sequences. These are all a genomic fingerprint analysis methods and widely used in the characterization of Firmicutes, Gram negatives and Actinomycetes[14,17,18]. These methods have been also used for studying the diversity in ecosystem, presenting the phylogenetic relation between strains, and discriminating the microorganism which are genetically close to each other[11,14,19].

The objective of this study was to identify and characterization *E. coli* O157:H7 strains isolated from meat samples, Erzurum, Turkey, by using phenotypic and genotypic methods.

MATERIALS AND METHODS

Bacterial Strain

Escherichia coli serotype O157:H7 ATCC 43894 was kindly provided by Sahan Guran at Firat University, Elazig, Turkey.

Isolation of *E. coli* O157

One hundred and forty meat samples were obtained from retail markets and butchers in Erzurum province, Turkey. The samples were carried to the laboratory and kept in a refrigerator. They

were used for isolation and identification of *E. coli* O157. Meat samples were collected into sterile plastic containers, transferred immediately to the laboratory at room temperature, and processed within 1 h after collection. For microbiological analysis, a 25 g sample was prepared by homogenizing with 225 ml EC Broth (Oxoid CM0990, England) containing novobiocin (0.02 gr ml^{-1}) (Oxoid, SR0181, England) in a stomacher (Laboratory Blender Stomacher 400, Seward Medical, London, UK) for 1 min and incubated at 37°C for 18 to 24 h[2]. 0.1 ml of samples are taken from pre-enriched culture and spread evenly on the CHROM-agar™ O157 (RTA, Kocaeli, Turkey) medium with a sterile spatula, left for incubation at 37°C for 18-24 hours. After incubation, different mauve colonies developed in the media were selected and purified by subculturing.

Extraction and analysis of fatty acid methyl ester (FAME) profiles

Preparation and analysis of FAME from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer‘s manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA)[20,21]. FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m × 0.2 mm) with cross-linked 5% phenyl methyl silicone. FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 60) with the MIS software package. The identity of bacterial strains was revealed by computer comparison of FAME profiles of the unknown test strains with those in the library.

Metabolic fingerprinting

One or two days before the inoculation of Biolog GN2 plates (Biolog), *E. coli* O157 strains were streaked on TSA plates. Each well of Biolog GN2 microtiter plates was inoculated with 125 µl of the Gram-negative bacterial suspension, respectively, adjusted to the appropriate density (108 cfu/ml) and incubated at 37 °C for 24 and 48 h. The development of colour was automatically recorded using a microplate reader with a 590 nm wavelength filter. Identification (Biolog Microloge 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/Databases/GN601 software[22].

Extraction of bacterial DNA from pure cultures

Total genomic DNA was extracted from bacteria samples using a modified method previously described by Adiguzel[13].

rep-PCR amplification

A total of 14 strains were subjected to rep-PCR genomic fingerprinting using primer sets corresponding to REP, ERIC, BOX and $(GTG)_5$ elements[14,17,23]. The 18-mer primer pair REP 1R (5'-IIIICGICGICATCIGGC-3') and REP 2 (5'-ICGICTTATCIGGCCTAC-3') (where I is Inosine); ERIC 1R (5'-ATGTAAGCT CCTGGGGAT-3') and ERIC 2 (5'-AAGTAAGTGACTGGGGGTGAGC-3'); BOX A1R (5'-CTACGGCAAGGCGACGCTGACG-3') and $(GTG)_5$ were used to amplify putative REP, ERIC- BOX- and $(GTG)_5$- like elements in bacterial DNA, respectively. Briefly, approximately 50 ng of purified DNA was used as a template in a 30 µl reaction mixture. 27 µl of reaction cocktail was prepared as follows: specify Gitschier Buffer 5 µl, Dimethyl sulfoxide 2.5 µl (100%, 20×), dNTPs (10 mM) 1.25 µl, bovine serum albumin 1.25 µl (20 mg/ml), primer/primers (5 µM) 3.0 µl, Taq polymerase (250 U) 0.3 µl, water 10.7 µl (for BOX and $(GTG)_5$ PCR, 13.7 µl). A negative control (no DNA) was included in each PCR assay. PCR amplification reactions were performed with a Corbett Research Palm Cycler (Corbett CG1-96 AG, Australia) using the following conditions: an initial denaturation at 95 °C for 7 min (94 °C, 7 min for $(GTG)_5$ primer); 30 cycles consisting of 94 °C for 1 min (94 °C, 30 s for $(GTG)_5$ primer) and annealing at 40, 45, 52 or 53 °C for 1 min with either REP, $(GTG)_5$, ERIC or BOX primers, respectively; extension at 65 °C for 8 min; and a sing1e final polymerization at 65 °C for 15 min before cooling at 4 °C. The PCR products (27 µl) were mixed with 6× gel loading buffer (3 µl) and subjected to agarose (1.5%w/v) gel electrophoresis in TAE (Tris–Acetate–EDTA) buffer at 90V for 120min. Amplification products, which were separated by gel, was stained in ethidium bromide solution (2 µl Etbr/100 ml 1× TAE buffer). The amplified DNA product was detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK).

The resulting fingerprints were transformed into a binary character matrix (‘1’ for the presence and ‘0’ for the absence of a band at a particular position) and analysed by using SPSS

program (SPSS, version 11.0 for Windows). Data were used to calculate a Jaccard (1908) similarity[24].

All of the experiments in this study were repeated at least twice

RESULTS AND DISCUSSION

E. coli O157:H7 is now a major cause of food-borne disease, in the United States, Canada, India, Thailand, Japan, Iran, Iraq, Turkey, Kenya, Argentina, Chile, Brazil and Europe[2,4,5,25,26].

A total 13 putative strains of *E. coli* O157 isolated from one hundred and forty meat samples were obtained from retail markets and butchers in Erzurum, were identified and characterized for the first time by comparing the REP, ERIC, BOX and $(GTG)_5$ PCR techniques, MIS and BIOLOG techniques.

Traditional phenotypic identification include the carbon source utilization system developed by Biolog, Inc., based on panels of biochemical reactions, and the gas-liquid chromatography system developed by MIDI, Inc. (Newark, Del.), based on the cellular fatty acid profile[27]. Profiling cellular fatty acids of microorganisms through using gas chromatography has long been reported as an important chemotaxonomic tool for the classification and identification of bacteria[28]. Fatty acid methyl ester analysis results showed that MIDI libraries were able to identify *E. coli* at species level. None of the test strains in the present study were identified at serotype level by MIDI. Therefore, our result demonstrated that MIDI libraries may not be strong enough for identification of *E. coli* at serotype level. They need to be improved. The characterization studies based on FAME analysis showed that total 20 different FAMEs were present in 13 bacterial strains tested in the present study (data not shown). Additionally, 9 of these fatty acids, 12:0, 14:0, 16:0, 17:0 w7c, 17:0 cyclo, 18:1 w7c, 18:1, 19:0 cyclo w8c, and 15: 0 iso 2OH fatty acids, were found in all isolates. Major fatty acids identified were 14:0, 16:0, 18:1w7c, and cyclo-nonadecanoic (19:0 cyclo) acids. Yuk *et al*.[3] found the similar major FAs in *E. coli* O157:H7 ATCC 43895. Wittaker *et al*.[29] determined 16:0, 17:0 w7c, and 19:0 w8c major FAs in *E. coli* O157:H7 strains. This shows that fatty acid profiles are strain-specific[30].

The Biolog bacterial identification system was used to identify the 13 Gram-negative isolates (using GN2 micro-plates) from 140 meat samples isolated after MIS and CHROM-agar™. The reference strain (*E. coli* O157:H7 ATCC 43894) included as control and identified using GN2 microplate to determine the specificity of the Microlog/Biolog microbial identification system. *E. coli* O157:H7 was identified up to *E. coli* species level. Similar finding related to the BIOLOG profiles

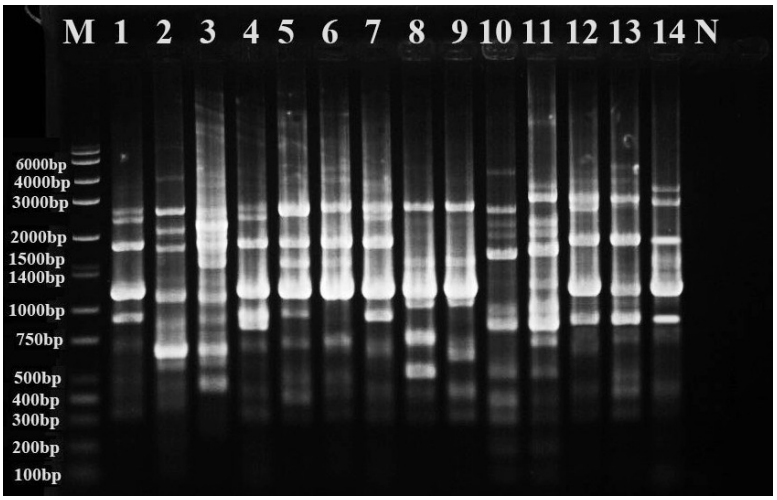

Fig. 1. ERIC-PCR profile generated with the ERIC 1R and ERIC 2 primers. Lanes: Lanes: 1) E1; 2) E2; 3) E3; 4) E4; 5) E5; 6) E6; 7) E7; 8) E8; 9) E9; 10) E10; 11) E11; 12) E12; 13) E13; 14) *E. coli* O157:H7 ATCC 43894 N; Negative Control; M) Molecular Marker (10 kb)

of *E. coli* O157:H7 serotype have been reported in the literature[31, 32].

The results show that MIS and Biolog identification systems alone are not yet accurate enough to serve as a primary method for identifying of *E. coli* O157:H7 serotype. According to our results, a single approach may not be sufficient for a reliable identification of *E. coli* O157:H7 strains.

Genotypic identification is emerging as an alternative or complement to established phenotypic methods[33]. Genomic fingerprints are the procedures of analyzing the whole genome of the targeted organisms. rep-PCR is one of the well-established genomic fingerprint methods applied for bacterial identification and characterization. The rep-PCR technique is simple, can differentiate between closely related strains of bacteria, and can assign bacteria potentially up to the strain level based on the presence of repeated elements within the genome examined[14].

In this study, two single oligonucleotide primers, BOXA1R and $(GTG)_5$, and two

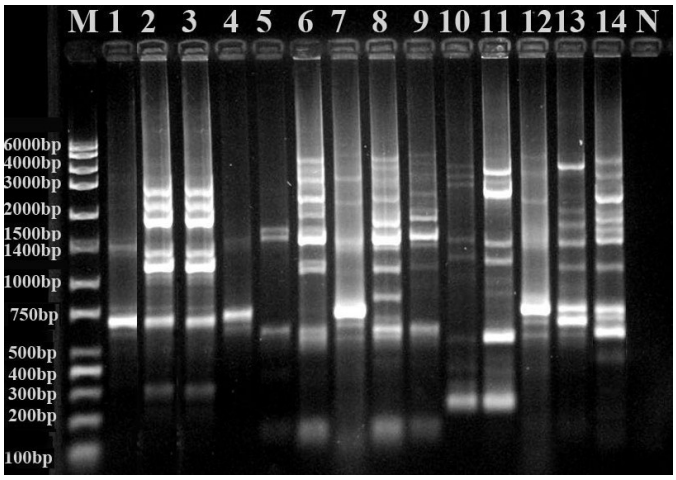

Fig. 2. REP-PCR profile generated with the REP 1R and REP 2 primers. Lanes: 1) E1; 2) E2; 3) E3; 4) E4; 5) E5; 6) E6; 7) E7; 8) E8; 9) E9; 10) E10; 11) E11; 12) E12; 13) E13; 14) *E. coli* O157:H7 ATCC 43894 N; Negative Control; M) Molecular Marker (10 kb)

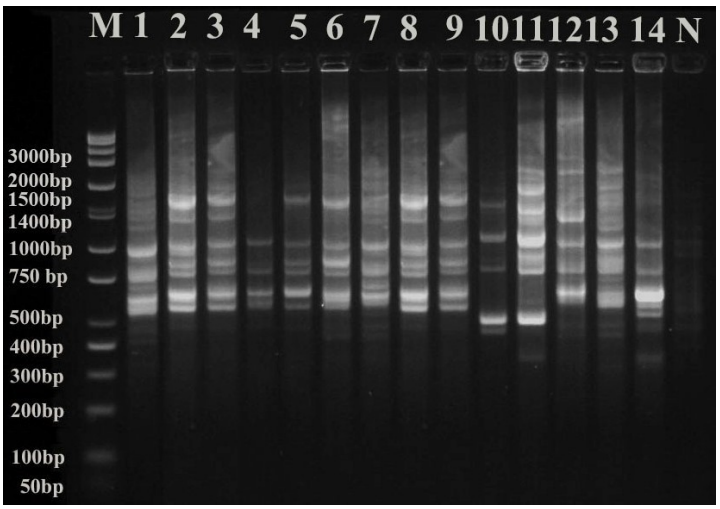

Fig. 3. BOX-PCR profile generated with the BOX A1 R primer. Lanes: 1) E1; 2) E2; 3) E3; 4) E4; 5) E5; 6) E6; 7) E7; 8) E8; 9) E9; 10) E10; 11) E11; 12) E12; 13) E13; 14) *E. coli* O157:H7 ATCC 43894 N; Negative Control; M) Molecular Marker (10 kb)

oligonucleotide primer pairs, REP1R-I/REP2-I and ERIC1R/ERIC2, were initially tested for their ability to type a subset of 13 strains. The ERIC1R/ERIC2 primer set significantly produced banding patterns with the highest complexity in comparison with the BOXA1R primer and the REP1R-I/REP2-I, $(GTG)_5$ primer sets. The ERIC primer set generated reproducible and differentiating fingerprints including 6-12 fragments of 250–4500 bp (Fig.1). The use of REP primers resulted in a banding pattern containing approximately 3–10 (200-4000bp) visualized PCR products (Fig.2). The BOX-PCR genomic fingerprints showed that bacterial strains have distinct patterns with 5–8 fragments in the size of 300–2000 bp and in

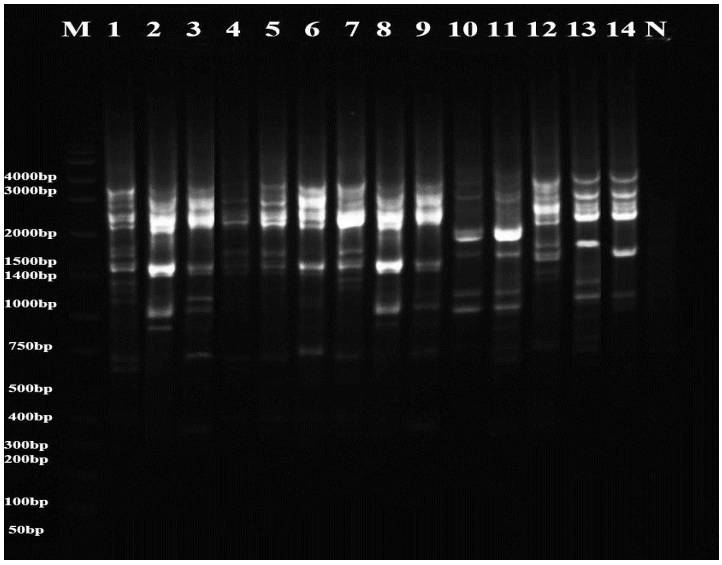

Fig. 4. GTG_5-PCR profile generated with the GTG_5 primer. Lanes: 1) E1; 2) E2; 3) E3; 4) E4; 5) E5; 6) E6; 7) E7; 8) E8; 9) E9; 10) E10; 11) E11; 12) E12; 13) E13; 14) *E. coli*

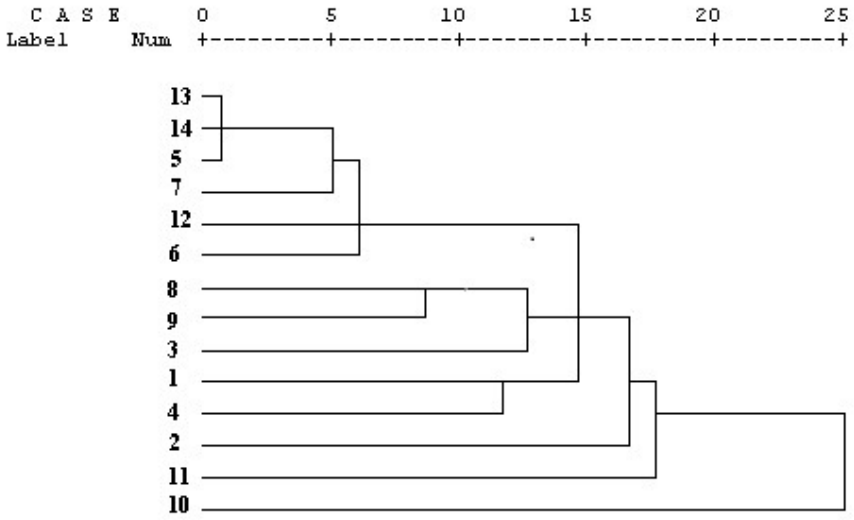

Fig. 5. ERIC-PCR Cluster Analyses: 1) E1; 2) E2; 3) E3; 4) E4; 5) E5; 6) E6; 7) E7; 8) E8; 9) E9; 10) E10; 11) E11; 12) E12; 13) E13; 14) *E. coli* O157:H7 ATCC 43894

frequently observed faint bands (Fig.3). The $(GTG)_5$ primer generated fingerprints containing between 6 and 11 fragments ranged from 500 to 3000 bp (Fig.4). Both methods (ERIC- and $(GTG)_5$-PCR) showed similar, almost identical, grouping of strains which demonstrated that these methods are reliable and suitable for discrimination of *E. coli* O157:H7 strains from other *E. coli* serotype strains. In general, the fingerprints, which were generated with the ERIC - PCR derived DNA fingerprints, showed the highest genetic polymorphism compared to $(GTG)_5$-, BOX- and REP fingerprints.

Some other studies reported similar results indicating that rep-PCR genomic fingerprint protocols exhibiting high sensitivity in the discrimination of *E. coli*[4, 33-37]. In their study, it was indicated that rep-PCR is an effective method for differentiating *E. coli* strains.

Due to the higher number of polymorphic bands produced by ERIC-PCR, this method was taking into account to do cluster analysis. *E. coli* O157 isolates were classified in two main clusters (Fig. 5). The first main cluster (E1-E9, E11-E13) was represented by twelve test strains and a reference strain (*E. coli* O157:H7 ATCC 43894), which were discriminated by two sub-clusters (Fig. 5). The results showed that 99 % of genetic relatedness among isolates E5, E13, *E. coli* O157:H7 ATCC 43894 was observed. Moreover, a high degree of similarity (≥82 %,) was found among twelve isolates (E1-E9, E12, E13, reference strain) and strain E 11 (Fig. 5). Second cluster was including one test strain (E 10) and a reference strain (14) of *E. coli* O157:H7, with low similarity ratio (≥75%).

Hahm *et al*.[38] showed that rep-PCR method was the easiest and quickest method that could be performed at a relatively low cost for discrimination of *E. coli* O157:H7 strains from other *E. coli* serotype strains.

Gevers *et al*.[23] assessed the applicability of rep-PCR fingerprinting for the genotypic differentiation of a broad range of *Lactobacillus* species. They found that the rep-PCR fingerprinting technique using $(GTG)_5$-PCR was the most suitable method for the identification of LAB. Adiguzel *et al*.[14] determined that the $(GTG)_5$-PCR method generated more informative results compared with ERIC-, REP- and BOX-PCR of *Geobacillus*, *Anoxybacillu*s and *Bacillus* spp.

Sahilah *et al*.[39] reported the genetic differences between the isolates belonging to *E. coli* species isolated from different source. Their findings showed that RAPD-PCR method to be less discriminatory than ERIC-PCR. Rice[40] concluded that $(GTG)_5$-PCR method is successful in showing the discrimination between *E. coli* strains recovered from different sources.

This is the first study that rep-PCR genomic fingerprint analysis methods, FAMEs and BIOLOG technics have been used to compare with each other for the molecular characterization of *E. coli* O157:H7 strains isolated from meat samples that were obtained from retail markets and butchers. The results in the present study demonstrated that the rep-PCR fingerprinting technique using $(GTG)_5$- and ERIC-PCR was a rapid, easy-to-perform, and reproducible tool for differentiation of *E. coli* O157:H7 at the serotype level, with a single-performance protocol. $(GTG)_5$- and ERIC-PCR fingerprinting methods could be a good choice for the genotypic characterization and phylogenetic analysis of *E. coli* O157:H7 strains. Therefore, present study results confirmed the previous studies[6,36,39,40] suggesting that rep-PCR fingerprinting is powerful molecular techniques not only for estimating genetic relatedness, but also for identification and characterization of *E. coli* O157:H7 strains (Figs. 1, 5).

ACKNOWLEDGEMENTS

This study was supported by grants from the Research Funds appropriated to Atatürk University.

REFERENCES

1. Tavakoli, H., Bayat, M., Kousha, A., Panahi, P. The Application of Chromogenic Culture Media for Rapid Detection of Food and Water Borne Pathogen. *American-Eurasian J. Agric.& Environ. Sci*., 2008; **4**: 693-698.
2. Vuddhakul, V., Patararungrong, N., Pungrasamee, P., Jitsurong, S., Morigaki, T., Asai, N., Nishibuchi, M. Isolation and characterization of *Escherichia coli* O157 from retail beef and bovine feces in Thailand. *FEMS Microbiol. Lett.*, 2000; **182**: 343-347.
3. Yuk, H.G., Marshall, D.L. Adaptation

of *Escherichia coli* O157:H7 to pH Alters Membrane Lipid Composition, Verotoxin Secretion, and Resistance to Simulated Gastric Fluid Acid. *Appl. Environ. Microbiol*., 2004; **70**: 3500-3505.

4. Maldonado, Y., Fiser, J.C., Nakatsu, C.H., Bhunial, A.K. Cytotoxicity Potential and Genotypic Characterization of *Escherichia coli* Isolates from Environmental and Food Sources. *Appl. Environ. Microb*., 2005; **71**: 1890–1898.
5. Jamshidi, A., Bassami, M.R., Rasooli, M. Isolation of *Escherichia coli* O157:H7 from ground beef samples collected from beef markets, using conventional culture and polymerase chain reaction in Mashhad, northeastern Iran. *Iran. J. Vet. Res*., 2008; **9**: 72-76.
6. Dini, C., De Urraza, P.J. Isolation and selection of coliphages as potential biocontrol agents of enterohemorrhagic and Shiga toxin-producing *E. coli* (EHEC and STEC) in cattle. *J. Appl. Microbiol*., 2010; **109**: 873-877.
7. Chapman, P.T. Methods available for the detection of Escherichia coli O157 in clinical, food and environmental samples, *World J. Microb. Biot*., 2000; **16**: 733-740.
8. Wallace, J.S., Jones, K. The use of selective and differential agars in the isolation of *Escherichia coli* O157 from dairy herds. *J. Appl. Bacterial*., 1996; **81**: 663-668.
9. De Boer, E., Heuvelink, A.E. Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli*. *J Appl Microbiol*. [Symp Suppl], 2000; **88**: 133-143.
10. Kimura, R., Mandrell, R.E., Galland, J.C., Hyatt, D., Riley, L.W. Restriction-site-specific PCR as a rapid test to detect enterohemorrhagic *Escherichia coli* O157: H7 strains in environmental samples. *Appl. Environ. Microbiol*., 2000; **66**: 2513-2519.
11. Adiguzel A. Molecular Characterization of thermophilic Bacteria Isolated from Water Samples Taken from Various thermal Plants. PhD, Atatürk University, Graduate School at Natural and Applied Sciences, Erzurum, Turkey, 2006; 130.
12. Zaliha, R.N., Rahman, R.A., Leow, T.C., Salleh, A.B., Basri, M. *Geobacillus zalihae* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. *BMC Microbiol*., 2007; **7**: 77.
13. Adiguzel, G. Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermente sausage. *Ph.D. Thesis*, Ataturk University, Graduate School of Health Sciences, Erzurum, Turkey, 2008; 110.
14. Adiguzel, A, Ozkan, H, Baris, O., Inan, K., Gulluce, M. Identifi cation and characterization of thermophilic bacteria isolated from hot springs in Turkey. *J. Microbiol Meth*., 2009; **79**: 321-328.
15. Adiguzel, A., Inan, K., Sahin, F., Arasoglu, T., Gulluce, M., Belduz, A.O., Baris, O. Molecular diversity of thermophilic bacteria isolated from Pasinler hot spring (Erzurum, Turkey). *Turk J Biol.*, 2011; **35**: 267-274.
16. Kotan, R., Sahin, F., Ala, A. Identification and pathogenicity of bacteria isolated from pome fruit trees in the Eastern Anatolia region of Turkey. *J. Plant Dis. Protect*., 2006; **113**: 8-13.
17. Versalovic, J., Schneider, M., De Bruijn, F.J., Lupski, J.R. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol. Cell. Biol*., 1994; **5**: 25-40.
18. Masco, L., Huys, G., Gevers, D., Verbrugghen, L., Swings, J. Identification of Bifidobacterium species using rep-PCR fingerprinting. *Syst. Appl. Microbiol*., 2003; **26**: 557–563.
19. Rademaker, J.L.W., De Bruijn, F.J. Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer-assisted pattern analysis. In: Caetano-Anolles, G., Gresshoff, P.M. (Eds.), DNA Markers: Protocols, Applications and Overviews. John Wiley & Sons, Inc, New York, USA, 1997, pp 1–26.
20. Miller, L.T., Berger, T. Bacteria identifi cation by gas chromatography of whole cell fatty acids. In: Hewlett-Packard Application Note 228–41. Hewlett-Packard, Avondale, Pennsylvania, 1985; 1-8.
21. Roy, A. Use of fatty acid for identification of phytopathogenic bacteria. *Plant Dis*., 1988; **72**: 460.
22. Bochner, B.R. Sleuthing out bacterial identities. *Nature*, 1989; **339**: 157-158.
23. Gevers, D., Huys, G., Swings, J. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol. Lett*., 2001; **205**: 31-36.
24. Yildiz, N., Bircan, H. Araþtýrma ve Deneme Metodlarý. Atatürk Üniversitesi Yay. No: 697, Ziraat Fak. Yay. Ders Kitaplarý Serisi No:57, Erzurum, Turkey, 1991.
25. Ulukanli, Z, Cavli, P., Tuzcu, M. Detection of *E. coli* O157:H7 from beef doner kebabs sold in Kars. *G. U. J. Sci*., 2006; **19**: 99-104.
26. Unsal, C. Occurance *E. coli* O157: H7 in meat consumed in Erzurum. *M.S. Thesis*, Ataturk University, Graduate School of Health Sciences, Erzurum, Turkey, 2007; 76.

27. Tang, Y.W., Ellis, N.M., Hopkins, M.K., Smith, D.H., Dodge, D.E., Persing, D.H. Comparison of Phenotypic and Genotypic Techniques for Identification of Unusual Aerobic Pathogenic Gram-Negative Bacilli. *J. Clin. Microbiol*., 1998; **36**: 3674–3679.
28. Kim, S.R., Kim, H.T., Park, H.J., Kim, S., Choi, H.J., Hwang, G.S., Yi, J.H., Ryu, D.H., Kim, K.H. Fatty acid proûling and proteomic analysis of *Salmonella enterica* serotype *typhimurium* inactivated with supercritical carbon dioxide. *Int. J. Food Microbiol*., 2009, **134**: 190–195.
29. Whittaker, P., Fry, F.S., Curtis, S.K., Al-Khaldi, S.F., Mossoba, M.M., Yurawecz, M.P., Dunkel, V.C. Use of fatty acid profiles to identify food-borne bacterial pathogens and aerobic endospore-forming bacilli. *J. Agric. Food Chem.*, 2005; **53**: 3735-3742.
30. Suutari, M., Laakso S. Microbial fatty acids and thermal adaptation. *Crit. Rev. Microbiol.*, 1994; **20:** 285–328.
31. Odumeru, J.A., Steele, M., Fruhner, L., Larkin, C., Jiang, J., Mann, E., Mcnab, W.B. Evaluation of Accuracy and Repeatability of Identification of Food-Borne Pathogens by Automated Bacterial Identification Systems. *J. Clin. Microbiol*., 1999; **37**: 944–949.
32. Muller, E.E., Ehlers, M.M. Biolog identification non-sorbitol fermenting bacteria isolated on *E. coli* O157:H7 selective CT-SMAC agar, *Water SA*., 2005; **31**: 247-251.
33. Mohapatra, B.R., Broersma, K., Nordin, R., Mazumder, A. Evaluation of repetitive extragenic palindromic-PCR for discrimination of fecal *Escherichia coli* from humans, and different domestic- and wild-animals. *Microbiol. Immunol*., 2007; **51**: 733-740.
34. Della-Costa, L.M., Irino, K., Rodrigues, J., Rivera, I.N.G., Trabulosi, L.R. Characterization of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR. *J. Med. Microbiol*., 1998; **47**: 227-234.
35. Da Silveira, W.D., Ferreira, A., Lancellotti, M., Barbosa, I.A., Leite, D.S., De Castro, A.F., Brocchi, M. Clonal relationships among avian *Escherichia coli* isolates determined by enterobacterial repetitive intergenic consensu (ERIC)-PCR. *Vet. Microbiol.,* 2002; **89**: 323-328.
36. Osek, J. Genetic Relatedness of *E. coli* O157:H7$^-$ Strains Isolated from Pigs Determined by Random Amplification of BOX DNA Sequences. *Bull. Vet. I. Pulway*, 2002; **46**: 3-10.
37. Wieczorek, K., Kowalczyk, A., Osek J. Relationship between phenotype and genotype of *Escherichia coli* O149:K91, F4 strains isolated from pigs with diarrhoea. *Bull. Vet. I. Pulway*, 2004; **48**: 219-223.
38. Hahm, B.K., Maldonado, Y., Schreiber, E., Bhunia, A.K., Nakatsu, C.H. Subtyping of foodborne and environmental isolates of *Escherichia coli* by multiplex-PCR, rep-PCR, PFGE, ribotyping and AFLP. *J. Microbiol Meth*., 2003; **53**: 387-399.
39. Sahilah, A.M., Audrey, L.Y.Y., Ong, S.L., Wan Sakeenah, W.N., Safiyyah, S., Norrakiah, A.S., Aminah, A., Ahmad Azuhairi, A. DNA profiling among egg and beef meat isolates of *Escherichia coli* by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR). *Int. Food Res. J*., 2010; **17**: 853-866.
40. Rice, W.C.: Design and evaluation of PCR primers which differentiate *Escherichia coli* O157:H7 and related serotypes. *J. Appl. Microbiol.,* 2009; **106**: 149-160.