JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2012. Vol. **6**(4), p. 1581-1589

Phenotyping and Genotyping Characterization of *Salmonella* Strains Isolated from Retail Beef in Erzurum, Turkey

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

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

(Received: 02 October 2012; accepted: 18 November 2012)

In this study, phenotypic and genotypic characterization of *Salmonella* strains was carried out. Isolates were obtained from 140 different minced beef samples, taken from slaughterhouses and butcher shops in the vicinity of Erzurum, by using chromogenic media. For this purpose, fatty acid profiles, BIOLOG, ERIC-PCR, REP-PCR and $(GTG)_5$-PCR methods were used. As a result of isolation study, *Salmonella* spp. was detected in 4 samples out of 140 minced beef samples. Depending on the data of fatty acid analysis, it was detected that there were 14 different fatty acids in the examined 15 bacterial strains. In the results of analyses by using BIOLOG system, all of the test organisms gave identification result as *Salmonella* group 1 and it was determined that this method is inefficient for serotype level detection. Several primer sets, targeting the repetitive DNA elements of REP, ERIC and $(GTG)_5$,were evaluated and it was detected that for identification and taxonomic characterization of *S. typhimurium* strains, the ERIC primers were the most reliable technique. Hence, as a promising genotypic tool, rep-PCR fingerprinting using the ERIC and REP primers can be used for the identification and characterization of *Salmonella* isolates species to serotype level.

Key words: *Salmonella* strains, Ground meat, MIS, BIOLOG, rep-PCR .

Foodborne bacterial pathogens are so important in terms of food safety. In addition to this, correctly identification of these pathogens has also a significant importance for both the food quality and the traceability of contamination factors which may occur in the supply chain[1].

In recent years, there is a considerable increase in foodborne diseases as a result of bacterial contamination of foods. Although there are different views of science people regarding the incidence of these diseases in the world, it is predicted that foodborne diseases are observed in about the 30% of the population of industrialized countries and, this percentage is even more in developing countries[2,3].

One of the most important worldwide public health issues is foodborne diseases, mainly caused by *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7 and

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

Yersinia spp. Above mentioned pathogenic bacteria have been present in different kinds of foods (fish, vegetables, dairy products, meat and meat products) related with outbreaks[2,4,5]. Among these pathogenic microorganisms, *Salmonella*, which is responsible for the significant part of foodborne diseases observed in human beings and animals, is a member of Enterobactericeae family. There are two species belonging to this microorganism group (*S. enterica* ve *S. bongori*) and, type species is *S. enterica*. In addition to its being the main cause of several infections and outbreaks around the world, it is counted as one of the significant reason for human gastroenteritis. It has been detected that *Salmonella*-contaminated food products, responsible for human salmonellosis, are mainly originated from cattle and poultry[6,7].

For studying foodborne pathogens, the conventional methods such as phage typing, serotyping, biotyping, antibiotic resistance (R-type), antibiogram, and bacteriocin identification are well known ones. These mostly unreliable methods are laborious, nonsensitive and, slow serotyping and phages typing, as conventional methods, are widely used to type *Salmonella.* Serotyping of bacteria depends on antigen-antibody interaction[8,9]. For *Salmonella,* there are 2 surface antigens, used to divide the bacteria into serogroups, flagella (H) and somatic (O). Up to now, over 2.500 *Salmonella* serovars have been identified in the world with respect to their O and H antigens[6,7]. Lysing bacteria with bacteriophages provides a basis for phage typing method. *Salmonella* serotypes such as *S. typhi* and *S. typhimurium* have a surface envelope (Vi) antigen which makes it possible to identify by using specific phages[9].

Molecular methods have been developed by taking into account the differences arising from nature, variations and ratios of macromolecules, which are forming microorganisms. Molecular methods use carbohydrates, lipids, proteins and genetic materials (DNA and RNA) as study materials. By using one of these items or a combination, identification and characterization of microorganisms are carried out[10].

In recent years, for identification of microorganisms, molecular methods is being intensely used like serologic techniques (Radioimmunoassay, Immunoblot, Enzyme-Linked Immunosorbent Assay, Dot Immunobinding Assay, Agglutination and Immunofluorescence), nucleic acid based analysis techniques (Randomly Amplified Polimorphic DNA= RAPD, Repetetive Extragenic Palindromic (rep-PCR), Enterobacterial Repetetive Intragenic Concensus (ERIC-PCR), BOX-PCR, $(GTG)_5$-PCR, Specific PCR, Restriction Fragment Length Polymorphism (RFLP), PCR-RFLP, Amplified Ribosomal DNA Restriction Analysis (ARDRA) and Denaturing Gradient Gel Electrophoresis (DGGE), determination of metabolic enzyme products (Biolog), fatty acids analyses (Microbial Identification System=MIS). Among these methods, MIS and BIOLOG, being completely computer controlled commercial systems, are used for identification and characterization of microorganisms in laboratories conducting routine tests[10,11].

First of these commercial systems is fatty acid methyl ester (FAMEs) which is an analysis depends on the identification of organisms with respect to fatty acid profiles. Besides its being an inexpensive and easily applicable method in a short time, this method compares the results by its own library by conducting qualitative and quantitative analyses with respect to fatty acid methyl esters of microorganism, grown in culture media. Furthermore, since it provides identifications and taxonomic classification of microorganisms, it is often used by scientists for identification purposes[12-15].

Another one is BIOLOG system. It is a molecular method, developed by Biolog. Inc. (Hayward, CA, USA), depends on identification of microorganisms with respect to their metabolic differences[16-18]. However, for the identification of many bacteria, this method is not found to be enough accurate to be used as a primary method, yet[5].

Additionally, primers, targeting non-coding repetitive sequences mixed throughout the fungi and bacteria genome, are used by the repetitive sequence-based PCR method. Lying close to the repeated elements, the conserved region shows differences with respect to size, thus producing fragments of varying length, evident via agarose gel electrophoresis. The size of fragment provides a distinct fingerprinting profile for the organism, which makes band comparison

possible. It is possible to identify several bacteria at the subspecies and strain level by rep-PCR[10, 19].

The purpose of this study is to carry out the phenotypic and genotypic characterization of *Salmonella* isolates, obtained from minced beef meats marketed in Erzurum city, by using FAMEs, BIOLOG, REP-, ERIC- and, $(GTG)_5$-PCR methods and, determination of which molecular method can successively be used in the identification of *Salmonella* isolates.

MATERIALS AND METHODS

Bacterial Strain

Salmonella thyphimurium ATCC 14028 was kindly provided by Sahan Guran at Firat University, Elazig, Turkey.

Isolation of *Salmonella* spp

One hundred and forty meat samples were obtained from retail markets and butchers in Erzurum province, Turkey. The samples were collected into sterile plastic containers and kept in a refrigerator. For detection of *Salmonella* spp., each 25 g meat sample was mixed with 225mL of sterile buffered peptone water (BPW, Merck KGaA, 107228, Germany) and pummeled in a Stomacher (Laboratory Blender Stomacher 400, Seward Medical, London, UK) apparatus for 2 minutes; the mixture was then incubated overnight at 37°C. A 0.1 volume of this culture was then transferred to 10 ml of Rappaport Vassiladis broth (RVS Broth, Merck KGaA, 107700, Germany) and incubated over night at 42 °C[2]. 0.1 ml of samples are taken from pre-enriched culture and spread evenly on the CHROM-agar™ *Salmonella* (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 FAME

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

Biolog Micro plate assay

One or two days after *Salmonella* spp. strains were streaked on TSA plates, the inoculation of Biolog GN2 plates (Biolog) was carried out. Each well of Biolog GN2 microtiter plates was inoculated with 125 µl of the Gram-negative bacterial suspension, respectively, and then, was adjusted to the proper density (108 cfu/ml) and incubated at 37 °C for 24 and 48 h. A microplate reader with a 590 nm wavelength filter was used to record automatically the development of color. By using BIOLOG420/Databases/GN601 software with applying the automatic threshold option, identification (Biolog Microlog 34.20 database) and ASCII file output of test results were conducted[5,22].

DNA extraction and polymerase chain reaction (PCR) amplification

A modified method, previously described by Adiguzel[23], was used for the extraction of total genomic DNA from bacteria samples.

rep-PCR

Using primer sets corresponding to REP, ERIC and $(GTG)_5$ elements, rep-PCR genomic fingerprinting was performed for a total of 15 strains[5,24,25]. For amplifying putative REP 1R (5'-IIIICGICGICATCIGGC-3') and REP 2 (5'-ICGICTTATCIGGCCTAC-3') (where I is Inosine); ERIC 1R (5'-ATGTAAGCTCCTGGGGAT-3') and ERIC 2 (5'-AAGTAAGTGACTGGGGGTGAGC-3') and $(GTG)_5$ were used, respectively. Concisely, as a template, approximately 50 ng of purified DNA was used 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 $(GTG)_5$ PCR, 13.7 µl). In each PCR assay, a negative control (no DNA) was included. A Corbett Research Palm Cycler (Corbett CG1-96 AG, Australia) was used to perform PCR amplification reaction under 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, or 52 °C for 1 min with either REP, $(GTG)_5$ or ERIC 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. 6× gel loading buffer (3 µl) was added to the PCR products (27 µl) and the mixture was subjected to agarose (1.5%w/v) gel electrophoresis in TAE (Tris–Acetate–EDTA) buffer at 90V for 120min. Amplification products, separated by gel, were stained in ethidium bromide solution (2 µl Etbr/100 ml 1× TAE buffer). The Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK) was used for the detection of the amplified DNA product.

All of the tests in this study were repeated at least twice.

Data analysis

A binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position) was formed with the resulting fingerprints and SPSS program (SPSS, version 11.0 for Windows) was used to analyze. Obtained data were used to calculate a Jaccard (1908) similarity[26].

RESULTS AND DISCUSSION

Foodborne diseases are responsible for the significant part of mortality and morbidity observed all around the world[27]. *Salmonella* spp., which is one of the main actors of these diseases, is being extensively isolated from raw meat, milk and milk products and poultry products[28].

Conventional methods for determination of foodborne pathogens take time since they need works to verify the data obtained from pre-enrichment, enrichment, purification by using selective solid culture media and, different biochemical test. Especially by means of important developments in biotechnology, in recent years, new analysis methods have been developed which are more specific, faster and more sensitive with respect to conventional methods[29]. Particularly, chromogenic culture media are widely used by scientists for isolation and identification of foodborne microorganisms[29-32]. In this research, by using CHROM-agar™ *Salmonella* (RTA, Kocaeli, Turkey) culture media, 15 *Salmonella* spp. have been isolated from 140 meat samples obtained from central Erzurum. Then, for the identification and characterization of the isolates, different phenotypic (MIS and BOLOG) and genotypic (REP, ERIC and $(GTG)_5$PCR) methods have been used. At first, as a result of applied FAMEs analysis, a total of 14 different fatty acid have been isolated from 15 bacterial strains. It has been detected that 8 fatty acids (12:0, 14:0, 16:0, 16:1 w9c, 17:1 w8c, 18:1 w7c, 18:1 w11c and 14:0 3OH) are common for all the isolates. Major fatty acids identified were 14:0, 16:0 and 18:1 w7c. Whittaker *et al*.[33] determined the similar major FAs in *S. typhimurium*. Aloui *et al*.[34] have found as a result of their study that 14:0, 16:0, 16:1w7 and 18:1w9 fatty acids in *S. enterica* serovar *typhimurium* strains are the major fatty acids. These data show that fatty acid profiles of organisms are specific to the strain[35].

For identifying the 15 Gram-negative isolates (using GN2 micro-plates) from 140 meat samples, isolated after MIS and CHROM-agar™, the Biolog bacterial identification system was used. *S. thyphimurium* ATCC 14028, the reference strain, included as control and was identified with GN2 microplate to figure out the specificity of the Microlog/Biolog microbial identification system. It was identified up to species level. The results of MIDI analysis were not similar to those obtained with the Biolog analyze for most cultures as it was denoted by Boulter *et al*.[36]. According to Jordan *et al*.[37], this technique is incapable of discriminating between different *Salmonella* serovars and could only be used as general detection for *Salmonella*. In the light of the results, it was concluded that for identifying *Salmonella* serotype, MIS and Biolog identification systems alone are not yet accurate enough to serve as a primary method. According to our results, a single approach may not be sufficiently reliable to identify Salmonella strains.

Genotypic identification is counted as an alternative or complement for the established phenotypic methods[38]. Genomic fingerprints are the procedures of analyzing the whole genome of the targeted organisms. For bacterial identification and characterization, one of the well-established genomic fingerprint methods is rep-PCR. As a simple technique, rep-PCR can provide differentiation between closely related strains of bacteria. Additionally, bacteria can be assigned up to the strain level by this technique based on the presence of repeated elements within the genome examined[25].

In this study, one single oligonucleotide primer, $(GTG)_5$, and two oligonucleotide primer pairs, REP1R-I/REP2-I and ERIC1R/ERIC2, were firstly tested to see whether they can type a subset of 15 strains or not. The ERIC1R/ERIC2 primer set notably gave banding patterns with the highest complexity with respect to the REP1R-I/REP2-I and $(GTG)_5$ primer sets. Including 10-16 fragments of 200–6000 bp (Fig.1), reproducible and differentiating fingerprints were obtained from the ERIC primer set generated. Using REP primers brought about a banding pattern containing approximately 7–9 (700-4000bp) visualized PCR products (Fig. 2). The $(GTG)_5$ primer generated fingerprints containing between 5 and 7 fragments ranged from 700 to 4000 bp (Fig.3). As a conclusion,

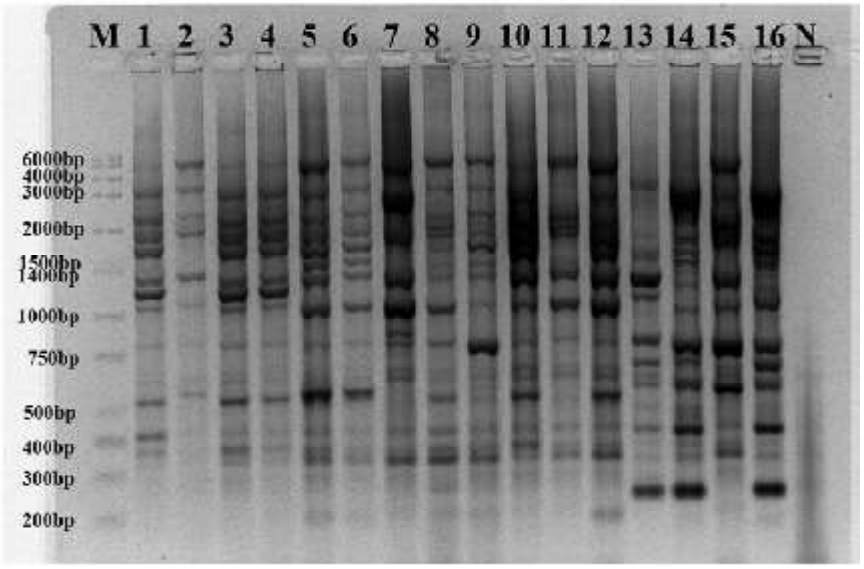

Fig. 1. ERIC-PCR profile generated with the ERIC 1R and ERIC 2 primers. Lanes: Lanes: 1) S1; 2) S2; 3) S3; 4) S4; 5) S5; 6) S6; 7) S7; 8) S8; 9) S9; 10) S10; 11) S11; 12) S12; 13) S13; 14) S14; 15) S15; 16) *S. thyphimurium* ATCC 14028; 17) N; Negative Control; M) Molecular Marker (10 kb)

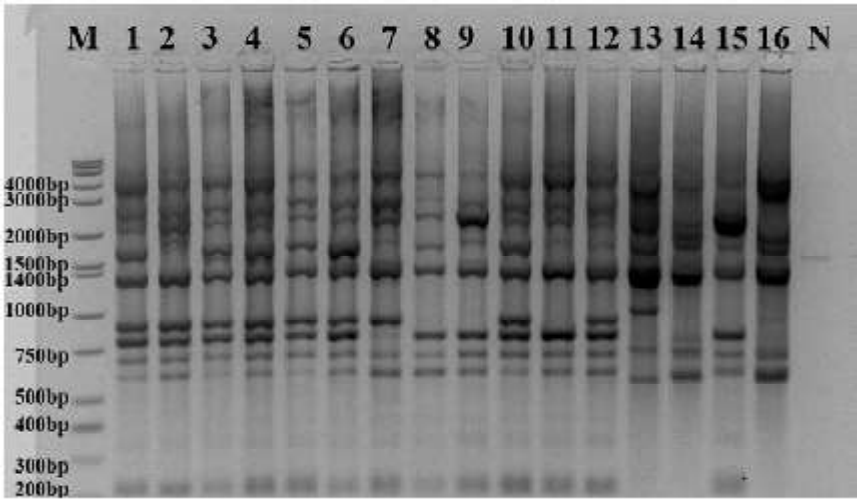

Fig. 2. REP-PCR profile generated with the REP 1R and REP 2 primers. Lanes: Lanes: 1) S1; 2) S2; 3) S3; 4) S4; 5) S5; 6) S6; 7) S7; 8) S8; 9) S9; 10) S10; 11) S11; 12) S12; 13) S13; 14) S14; 15) S15; 16) *S. thyphimurium* ATCC 14028; 17) N; Negative Control; M) Molecular Marker (10 kb)

fingerprints, generated with the ERIC -PCR derived DNA fingerprints, exhibited the highest genetic polymorphism compared to $(GTG)_5$-, and REP-PCR fingerprints.

Similar results have been reported in some other studies indicating that rep-PCR genomic fingerprint protocols demonstrating high sensitivity in the discrimination of *Salmonella* isolates[7,9,39-41]. It was also indicated in their study that rep-PCR is an efficient method for differentiating *Salmonella* strains.

In their study, Anderson *et al*.,[9] examined the applicability of DGGE (Denaturing Gradient Gel Electrophoresis) and REP-PCR methods in *Salmonella* serotyping with respect to conventional antibody serotyping method, which is used as golden standard. They stated that REP-PCR method forms more discriminating fragments

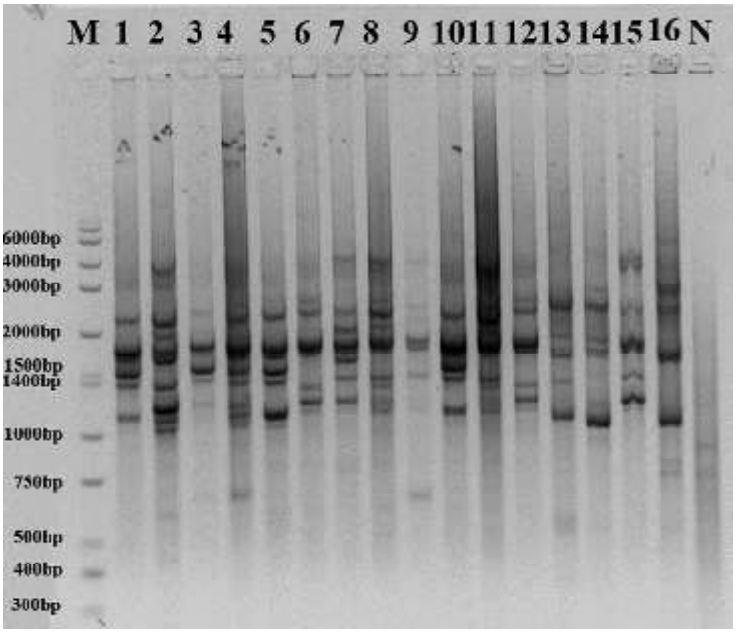

Fig. 3. GTG_5-PCR profile generated with the GTG_5 primer. Lanes: 1) S1; 2) S2; 3) S3; 4) S4; 5) S5; 6) S6; 7) S7; 8) S8; 9) S9; 10) S10; 11) S11; 12) S12; 13) S13; 14) S14; 15) S15; 16) *S. thyphimurium* ATCC 14028; 17) N; Negative Control; M) Molecular Marker (10 kb)

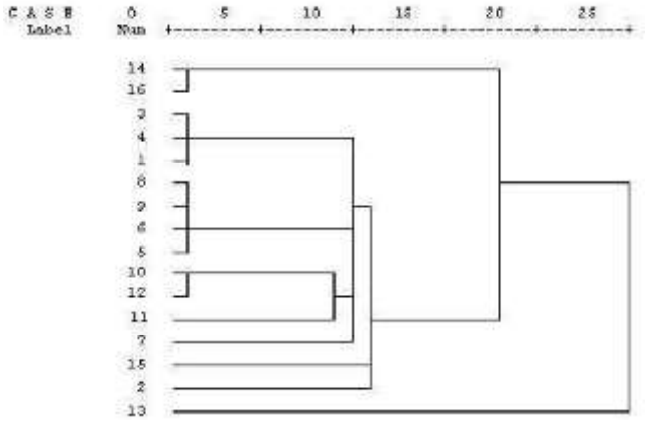

Fig. 4. 1) S1; 2) S2; 3) S3; 4) S4; 5) S5; 6) S6; 7) S7; 8) S8; 9) S9; 10) S10; 11) S11; 12) S12; 13) S13; 14) S14; 15) S15; 16) *S. thyphimurium* ATCC 14028

with respect to DGGE and also, both methods can be used as an alternative for the conventional antibody method.

Performing cluster analysis was taken into account because of the higher number of polymorphic bands produced by ERIC-PCR. *Salmonella* isolates were classified in two main clusters (Fig. 4). Fourteen test strains and a reference strain (*S. thyphimurium* ATCC 14028), which were discriminated by two sub-clusters (Fig. 4), represent the first main cluster (S1-S12, S14 and S15). It was detected that there was a 99 % of genetic relatedness between isolates S14 and *S. thyphimurium* ATCC 14028. Furthermore, there was a high degree of similarity (≥82 %,) among twelve isolates (S1-S12, S14 and S15) and a reference strain (Fig.4). Second cluster, represented by one test strain (S13) and a reference strain[16] of *S. thyphimurium* ATCC 14028, had low similarity ratio (≥75%), respectively.

Burr *et al*.,[42] assessed the applicability of rep-PCR fingerprinting for the genotypic differentiation of a broad range of *Salmonella* isolates. They found that the REP-PCR was able to discriminate among *Salmonella* isolates sharing similar serotypes. Gallardo *et al.*[43] suggested that REP-PCR method provided an alternative, rapid and powerful genomic typing method for S. *typhimurium*. Woo and Lee[44] reported that the REP-PCR method may represent an efficient and time-saving analysis tool for the genotyping of *Salmonella* serotypes, including *S. typhimurium.* Albufera *et al.,*[6] compared RAPD with rep-PCR for determination of genetic diversity of *Salmonella* isolates and determined that rep-PCR had greater discriminatory power than RAPD method. Li *et al*.[7] showed that ERIC-PCR method was an alternative, rapid and powerful genomic typing method for discrimination of *Salmonella* serotypes**.**

FAMEs, rep-PCR genomic fingerprint analysis methods and BIOLOG technics have been firstly used in this study to compare with each other for the molecular characterization of *Salmonella* strains isolated from meat samples that were obtained from retail markets and butchers. The results in this study indicated that the rep-PCR fingerprinting technique, using REP- and ERIC-PCR, was a rapid, easy-to-perform and, reproducible tool for differentiation of *Salmonella* strains at the serotype level, with a single-performance protocol. For the genotypic characterization and phylogenetic analysis of *Salmonella* strains, REP- and ERIC-PCR fingerprinting methods could be a good choice. Therefore, our results endorsed the previous studies[7,42-44] suggesting that rep-PCR fingerprinting is powerful molecular technique for both estimating genetic relatedness and for identification and characterization of *Salmonella* isolates (Figs. 1, 4).

ACKNOWLEDGMENTS

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

REFERENCES

1. 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.
2. Cloak, O.M., Duffy, G., Sheridan, J.J., Blair, I.S., Mcdowell,D.A.Isolation and detection of *Listeria* spp, *Salmonella* spp and *Yersinia* spp using a simultaneous enrichment step followed by a surface adhesion immunofluorescent technique. *J. Microbiol. Methods.,* 1999; **39**: 33-43.
3. Mead, P.S., Slutsker, L., Dietz, V., Mccaig, L.F., Bresee, J.S., Shapiro, C., Griffin,P.M., Tauxe, R.V. Food-Related Ilness and Death in the United States, *Emeg. Infect. Dis.*, 1999; **5**: 607-625.
4. Kawasaki, S., Fratamico, P.M., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., Kawamoto, S. Evaluation of A Multiplex PCR System for Simultaneous Detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Foods and in Food Subjected to Freezing. *Foodborne Pathog. Dis*., 2009; **6**: 81-89.
5. Adiguzel, G., Gulluce, M., Bozoglu, C., Yanmis, D., Gormez, A., Atasever, M., Adiguzel, A. Molecular Characterization of *Escherichia coli* O157:H7 from retail beef in Erzurum, Turkey. *J Pure Appl Microbiol*., 2012; **6**(3): 1033-1041.
6. Albufera, U., Bhugaloo-Vial, P., Issack, M.I., Jaufeerally-Fakim, Y. Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD

analysis. *Infect. Genet. Evol*., 2009; **9**: 322-327.
7. Li, Y., Fang, Y.H., Zhu, H.W., Sun, P., Wei, J.Z., Yin, Z.J. The Analysis of ERIC-PCR Genomic Polymorphism of *Salmonella* Isolated Strains in Pig Carcass. *J. Anim. Vet. Adv*., 2011; **10**: 1694-1698.
8. Sader, H.S., Hollis, R.J., Pfaller, M.A. The use of Molecular Techniques in the Epidemiology and Control of Infectious-Diseases. *Clin. Lab. Med*., 1995; **15**: 407-431.
9. Anderson, P.N., Hume, M.E., Bryd, J.A., Hernandez, C., Stevens, S.M., Stringfellow, K., Caldwell, D.J. Evaluation of repetitive extragenic palindromic-polymerase chain reaction and denatured gradient gel electrophoresis in identifying *Salmonella* serotypes isolated from processed turkeys. *Poult. Sci*., 2010; **89**: 1293-1300.
10. 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.
11. Rusznyak, A., Vladar, P., Szabo, G., Marialigeti, K., Borsodi, A.K. Phylogenetic and metabolic bacterial diversity of Phragmites australis periphyton communities in two Hungarian soda ponds. *Extremophiles*, 2008; **12**, 763–773.
12. Sasser, M. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. MIDI, Technical Note, 1990; 101.
13. Muller, K.D., Schmid, E.N., Kroppenstedt, R.M. Improved Identification of Mycobacteria by Using the Microbial Identification System in Combination with Additional Trimethyl-sulfonium Hydroxide Pyrolysis. *J. Clin. Microbiol*., 1998; **36**: 2477-2480.
14. Ritchie, N.J., Schutter, M.E., Dick, R.P., Myrold, D.D. Use of Length Heterogeneity PCR and Fatty Acid Methyl Ester Profiles to Characterize Microbial Communities in Soil. *Appl. Environ. Microbiol.*, 2000; **66**: 1668-1675.
15. Buyer, J.S. Identification of Bacteria from Single Colonies by Fatty Acid Ananlysis. *J. Microbiol. Meth*., 2002; **48**: 259-265.
16. Miller, J.M., Biddle, J.W., Quenzer, V.K., Mclaughlin, J.C.Evaluation of Biolog for identification of members of the family *Micrococcaceae. J. Clin. Microbiol*., 1993; **31**: 3170-3173.
17. Verniere, C., Pruvost, O., Civerolo, E.L., Gambin, O., Jacquemoud-Collet, J.P., Luisetti, **J.** Evaluation of the biolog substrate utilization system to identify and assess metabolic variation among strains of *Xanthomonas campestris* pv. *citri*. *Appl. Environ. Microb*., 1993 ; **59**: 243-249.
18. Konopka, A., Oliver, L., Turco, R.F. The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microb. Ecol*., 1998; **35**: 103-115.
19. Wise, M.G., Siragusa, G.R., Plumblee, J., Healy, M., Cray, P.J., Seal, B.S. Predicting *Salmonella enterica* serotypes by repetitive sequence-based PCR, *J. Microbiol. Meth*., 2009; **76:** 18–24.
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. 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.
24. 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.
25. 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.
26. Yildiz, N., Bircan, H. Araþtýrma ve Deneme Metodlarý. Atatürk Üniversitesi Yay. No: 697, Ziraat Fak. Yay. Ders Kitaplarý Serisi No:57, Erzurum, Turkey, 1991.
27. Smith, S.I., Fowora, M.A., Goodluck, H.A., Nwaokorie, F.O., Aboaba, O.O., Opere, B. Molecular typing of *Salmonella* spp isolated from food handlers and animals in Nigeria. *Int J Mol Epidemiol Genet*., 2011, **2**: 73–77.
28. Gorman, R., Adley, C.C.Characterization of *Salmonella enterica* serotype *typhimurium* isolates from human, food and animal sources in the Republic of Ireland. *J. Clin. Microbiol.,* 2004; **42**: 2314–2316.
29. 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.
30. Cassar, R., Cuschieri, P. Comparison of *Salmonella* Chromogenic Medium with DCLS

Agar for Isolation of *Salmonella* species from Stool Specimens. *J. Clin Microbiol.* 2003; **41**: 3229–3232.

31. Taquchi S., Nakamura, P. Chromagar MRSA: A technique for detection of resistance *S.aureus* species to methicilline. *J. of Jap. Ass. Inf. Dis.,*2005; 54-59.
32. van Dijk, S., Bruins, M.J., Ruijs, H.M. Evaluation and Implementation of a Chromogenic Agar Medium for *Salmonella* Detection in Stool in Routine Laboratory Diagnostics. *J. Clin Microbiol*.,2008; **47**: 456–458.
33. 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.
34. Aloui, A., Mihoub, M.,Sethom, M.M., Chatti,A., Feki, M., Kaabachi, N., Landoulsi, A. Effects of dam and/or seqA mutations on the fatty acid and phospholipid membrane composition of *Salmonella enterica* serovar *typhimurium*. *Foodborne Pathog Dis*., 2010; **7**: 573-583.
35. Suutari, M., Laakso,S.Microbial fatty acids and thermal adaptation. *Crit. Rev. Microbiol.*, 1994; **20:**285–328.
36. Boulter, J.I., Trevors, J.T.,Boland, G.J.Microbial studies of compost: bacterial identiûcation, and their potential for turfgrass pathogen suppression. *World J. Microb. Biot*., 2002; **18**: 661–671.
37. Jordan, R., van Heerden, E., Hugo, C.J., Piater, L.A. Using current molecular techniques for rapid differentiation of *Salmonella typhi* and *Salmonella typhimurium*. *Afr. J. Biotechnol*., 2009; **8**: 1815-1818.
38. 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.
39. Lim, H., Lee, K.H., Hong, C.H., Bahk, G.J., Choi, W.S. Comparison of four molecular typing methods for the differentiation of *Salmonella* spp. *Int. J. Food Microbiol*., 2005; **105**: 411 – 418.
40. Rasschaert, G., Houf, K., Imberechts, H., Grijspeerdt, K., De Zutter, L., Heyndrickx, M. Comparison of Five Repetitive-Sequence-Based PCR Typing Methods for Molecular Discrimination of *Salmonella enterica* Isolates. *J. Clin. Microbiol*., 2005; **43**: 3615-3623.
41. Foley, S.L., White, D.G.,Mcdermott, P.F., Walker, R.D., Rhodes, B., Fedorka-Cray, P.J., Simjee, S., Zhao, S. Comparison of Subtyping Methods for Differentiating *Salmonella enterica* Serovar *typhimurium* Isolates Obtained from Food Animal Sources. *J. Clin. Microbiol*.,2006; **44**: 3569-3577.
42. Burr, M.D., Josephson,K.L.,Pepper, L.L.An evaluation of ERIC-PCR and AP-PCR fingerprinting for discriminating *Salmonella* serotypes. *Lett. Appl. Microbiol*., 1998; **27**: 24-30.
43. Gallardo, F., Ruiz,J.,Marco, F., Towner,K.J., Vila, J. Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella* serotype *typhimurium* with investigation of molecular epidemiology and mechanisms of resistance. *J. Med. Microbial*., 1999; **48**: 367-374.
44. Woo,Y.K., Lee, S.H. Genetic Diversity of Multi-resistant *Salmonella enterica* Serotype t*yphimurium* Isolates from Animals and Humans. *The Journal of Microbiology*, 2006; **44**: 106-112.