Differentiation of *Salmonella* Typhimurium from *Salmonella* Enteritidis and other *Salmonella* Serotypes using Random Amplified Polymorphic DNA Analysis

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ABSTRACT *Salmonella enterica* ssp. *enterica* serovar Typhimurium and *Salmonella enterica* ssp. *enterica* serovar Enteritidis are the major dominating serotypes of *Salmonella* in poultry and poultry products. Infection by *Salmonella* Typhimurium is an important cause of morbidity and mortality in poultry. Rapid differentiation of *Salmonella* Typhimurium from other *Salmonella* serotypes including *Salmonella* Enteritidis can be very crutial for public health and for epidemiologists and for the poultry industry. Ten arbitrarily designed short primers (8 to 10 bases) were used in the random amplified polymorphic DNA analysis of *Salmonella* Typhimurium. One of the primers, primer 3 (5′-CGT GCA CGC-3′), resulted in the amplification of a band pattern that was unique to *Salmonella* Typhimurium. In total, 24 strains of serotype *Salmonella* Typhimurium were used during the study. Eighteen of them are clinical isolates, 2 of them chicken isolates (A6, A20), 2 of them from the Pasteur Institute, 1 from Refik Saydam National Culture Collection, and 1 is a type culture strain from National Culture Type Collection. Serotype *Salmonella* Typhimurium strains, which were collected from several different hospitals, institutes, and culture collections, have all displayed the same amplification band by primer 3. Twenty-three strains of 16 different serotypes of salmoneallae including 11 *Salmonella* Enteritidis strains gave only a 300-bp amplification band or no bands, whereas an additional 700-bp amplification band was observed only in samples of *Salmonella* Typhimurium serotype. It is concluded that random amplified polymorphic DNA analysis with primer 3 is of potential use as a serotype-specific marker for *Salmonella* Typhimurium.

Key words: *Salmonella* Typhimurium, *Salmonella* Enteritidis, random amplified polymorphic deoxyribonucleic acid analysis

2008 Poultry Science 87:1068–1074
doi:10.3382/ps.2007-00355

INTRODUCTION

Salmonella food poisoning continues to pose serious health problems, and studies toward finding rapid and sensitive tests for the detection and identification of *Salmonella* serotypes are therefore of great concern. *Salmonella* food poisoning results from the ingestion of viable cells of a member of the genus *Salmonella* and can be prevented to a great extent by adequate cooking, suitable refrigeration, protection of foods from contamination, and good personal sanitary and hygienic practices. Yet, the examination of food products for the presence of *Salmonella* is essential to prevent infections, and therefore, rapid tests are required to prevent prolonged storages of food products.

Serotype identification is necessary for investigations of foodborne outbreaks and provides the examination of the overall changes in the prevalance of a particular serotype. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the 2 most common *Salmonella* food-poisoning serotypes in the United Kingdom (Plummer et al.,1995). It was recently reported that most of the clinical isolates of foodborne diseases from different hospitals in Ankara, Turkey, were *Salmonella* Typhimurium (Yıldırmak et al., 1998). Although serotyping has shown that *Salmonella* Enteritidis displaced *Salmonella* Typhimurium as the most frequently isolated serotype, the identification of *Salmonella* Typhimurium serotype is still important in some countries (Eley, 1996) .

Methods for the detection of *Salmonella* include the conventional culture method, rapid screening methods, immunological methods, and DNA-based methods (Feng, 1992). The techniques used for the detection of *Salmonella* mostly suffer from being either time-consuming, labor intensive, or expensive. The application of the PCR is one approach for the rapid and effective detection and identification of salmonellae (Cano et al., 1993; Way et al., 1993; Bej et al., 1994; Van Lith and Aarts, 1994). In most of these studies, PCR amplifications have been based

Received August 23, 2007.
Accepted February 14, 2008.
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Table 1. *Salmonella* and non-*Salmonella* strains used in random amplified polymorphic DNA analysis

Salmonella spp.	Non-*Salmonella* species
Salmonella Typhimurium	*Escherichia coli*
Salmonella Enteritidis	*Klebsiella* sp.
Salmonella Typhi	*Pseudomonas fluorescens*
Salmonella Paratyphi B	*Pseudomonas aerogenes*
Salmonella Rissen	
Salmonella Baildon	
Salmonella Anatum (*rif*)	
Salmonella Anatum (*exconj*)	
Salmonella D group	
Salmonella B group	
Salmonella Orion	
Salmonella Kedougou	
Salmonella Quakam	
Salmonella Glostrup	
Salmonella Strasbourg	
Salmonella Bahrenfeld	
Salmonella Agona	
Salmonella Baildon	
Salmonella Havana	

Table 2. Arbitrarily designed primers used in random amplified polymorphic DNA analysis

Name of primer	Sequence of primer
Primer 1 (8 mer)	5′-GCC GAG CG-3′
Primer 2 (8 mer)	5′-CTG AGC GC-3′
Primer 3 (9 mer)	5′-CGT GCA CGC-3′
Primer 4 (9 mer)	5′-GAC GCC GTG-3′
Primer 5 (9 mer)	5′-CAG TCA GCG-3′
Primer 6 (9 mer)	5′-GCA N[1]GT CGC-3′
Primer 7 (10 mer)	5′-TCA CGA TGC A-3′
Primer 8 (10 mer)	5′-ACT GAT CAG G-3′
Primer 9 (10 mer)	5′-GCA NTG CGC T-3′
Primer 10 (10 mer)	5′-CGG TCA GTC C-3′

[1]N; all 4 bases; A, C, G, or T.

on prior sequence information from cloned genes (Rahn et al., 1992; Aabo et al., 1993, 1995; Cohen et al., 1996). The application of random amplified polymorphic DNA (**RAPD**) analysis (Welsh and McClelland, 1990; Williams et al., 1990; Caetano-Anolles et al., 1991) based on the random amplification of genomic DNA fragments through short arbitrarily designed primers is an attractive alternative for the detection and identification of microorganisms (Lawrence et al., 1993; Meunier and Grimont, 1993; Cave et al., 1994; Martinez-Murcia and Rodriquez-Valera, 1994; Stephan, 1996), especially where previous sequence information is not available. Random amplified polymorphic DNA analysis has the potential to detect polymorphism throughout the entire genome as compared other techniques. It also allows one to start a blind walk through the genomic DNA of an organism and to possibly discover regions of interest that would otherwise not be easily established. Furthermore, RAPD is more suitable than other techniques, because it does not require prior knowledge of target DNA and it uses short (9 to 10 bases) primers with a small amount of starting DNA. *Salmonella* Typhimurium together with *Salmonella* Enteritidis are the most prevalent serotypes of food poisoning all over the world, and this study was performed for the development of serotype-specific primer for one of those organisms. Thus, although RAPD is mainly used for molecular typing studies of bacteria, it is selected to differentiate *Salmonella* Typhimurium from other *Salmonella* serotypes in this study. The main objective of this study was to differentiate *Salmonella* Typhimurium from *Salmonella* Enteritidis that show very similar disease symptoms and also from other *Salmonella* serotypes using RAPD-PCR.

MATERIALS AND METHODS

Bacterial Strains

Salmonella and non-*Salmonella* species used in this work are presented in Table 1. These bacterial strains (Yıldırmak et al., 1998; İçgen et al., 2002a,b) were kindly provided by the Numune Hospital Department of Bacteriology, Ankara, Turkey; Uludağ University Faculty of Medicine, Bursa, Turkey; Department of Biology, Middle East Technical University, Ankara, Turkey; Department of Food Engineering, Middle East Technical University, Ankara Turkey; Faculty of Agriculture, Department of Food Science and Technology, Ankara University, Ankara, Turkey; Refik Saydam National Hygiene Center (Refik Saydam National Culture Collection), Ankara, Turkey; Institute of Health Protection, Ankara, Turkey.

Cultivation and Maintenance of Bacterial Strains

All *Salmonella* strains were grown in tryptic soy broth at 37°C for 1 d. Cultures were stored on tryptic soy agar slants at 4°C. For longer periods of maintenance, bacterial strains were kept in 20% glycerol and microbank at –80°C.

Before DNA isolation, optical density measurements were performed to analyze the growth state of the cultures according to previously determined growth curves. In general, an optical density measurement of 1.4 to 2.0 at 420 nm was found to yield efficient amplifications with all the different bacterial species tested, and optical density at 420 nm = 1.9 to 2.0 was determined as optimal for the preparation of template DNA from *Salmonella* spp. tested. In general, *Salmonella* spp. reach the logarithmic phase of growth within 6 to 7 h, which is found to be the stage to give the best quality DNA for PCR amplifications. This corresponds to a concentration between 3.5×10^9 and 8×10^9 cfu/mL, as determined by serial dilutions and total plate count assay on tryptic soy agar plates.

Preparation of Template DNA for PCR

Isolation of genomic DNA was performed by the modified method of Maloy (1990). According to this method, bacterial cells from an overnight culture previously grown at 37°C in 10 mL of tryptic soy broth were transferred to 1.5-mL Eppendorf tubes and spun at 18,000 × *g* for 2 min. After supernatant was removed, a new 1.5-mL cell culture was added onto the pellet and again spun at 18,000 × *g* for 2 min. Then, the pellet was resuspended

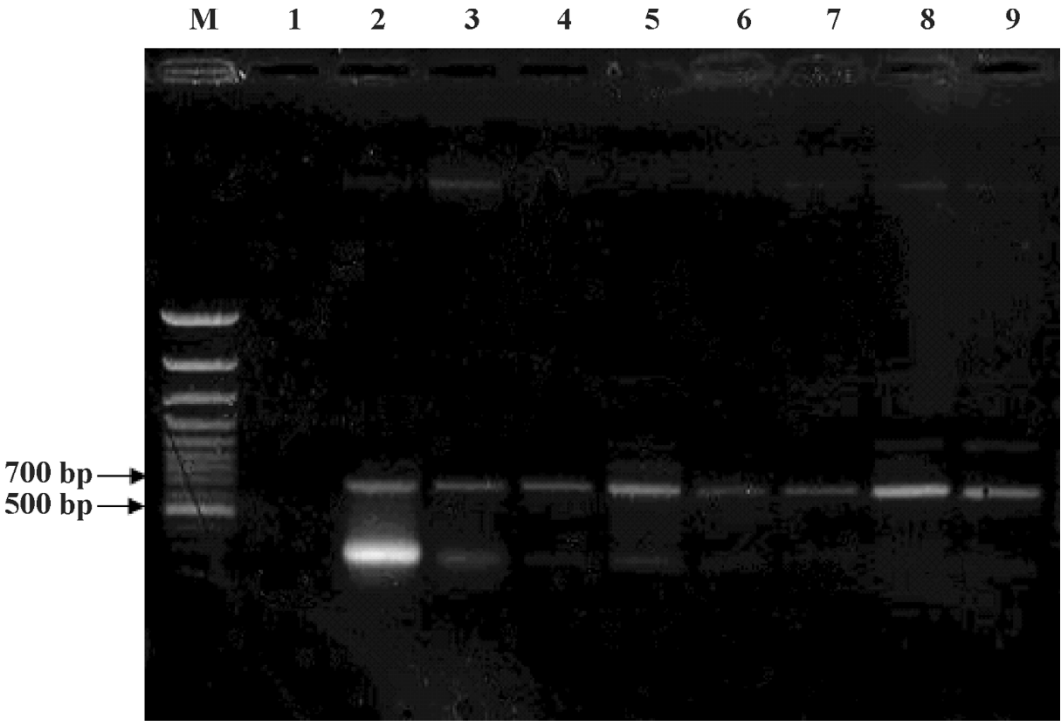

Figure 1. Amplification results of *Salmonella* Typhimurium; M = 100-bp DNA ladder plus marker; 1 = negative control; 2 = *Salmonella* Typhimurium A6; 3 = *Salmonella* Typhimurium A20; 4 = *Salmonella* Typhimurium H28; 5 = *Salmonella* Typhimurium H29; 6 = *Salmonella* Typhimurium H31; 7 = *Salmonella* Typhimurium H33; 8 = *Salmonella* Typhimurium H49; 9 = *Salmonella* Typhimurium ST4.

in 467 μL of Tris-EDTA buffer by repeated pipetting, 30 μL of 10% SDS, and 3 μL of 20 mg/mL of proteinase K were added, and this mixture was incubated at 37°C for 1 h. Following 1 h of incubation, an equal volume (500 μL) of phenol-chloroform-isoamylalcohol was added and mixed well by inverting the tube until the phases were completely mixed, and then the tube was spun at 18,000 × *g* for 2 min. The upper aqueous phase containing nucleic acids was carefully transferred to a new tube, and phenol-chloroform-isoamylalcohol extraction was repeated until the interphase was clear. The aqueous phase obtained after the last in the series of deproteinization was mixed with 0.1× volume of 3 *M* sodium acetate, and ethanol precipitation was performed by adding 2× volume of ice-cold 95% ethanol. The DNA was precipitated overnight at –20°C and collected by centrifugation at 25,000 × *g* for 15 min. Finally, the supernatant was discarded, the pellet was washed with 1 mL of ice-cold 70% ethanol to eliminate salt, and centrifuged another 30 s at 25,000 × *g*. The tube was allowed to air dry until all the ethanol was evaporated. The DNA was dissolved in 30 to 50 μL of Tris-EDTA buffer and stored at –20°C.

The resulting DNA concentration was determined spectrophotometrically and also by comparing the intensity of the band with that of the bands of known concentration on agarose gel.

PCR Amplifications

A 50-μL PCR mixture contained 50 ng of template DNA, 2 m*M* deoxynucleoside triphosphate mix, 200 pmol of primer, 2 U of Taq DNA polymerase (MBI Fermentas Vilnius, Lithuania) in 1× reaction buffer [50 m*M* KCl, 10 m*M* Tris-HCl (pH 8.8), 0.8% Nonidet P40, 1.5 m*M* $MgCl_2$]. The reaction mixtures were overlaid with 30 μL of mineral oil. The PCR cycles consisted of the following steps: 1 × 90°C, 5 min; 35 × [89°C, 1 min; 32°C, 1 min; 72°C, 1.5 min]; and 1 × 50°C, 3 min. Plasmid DNA with sequence-specific primers was used as a positive control to check the proper functioning of the enzyme and other reaction components as well as the PCR machine. No DNA negative controls with one of the arbitrary primers were also performed to check for possible contaminations. Arbitrarily designed short primers 8 to 10 bases long used for PCR amplifications are shown in Table 2. The primer design considerations were the incorporation of 50 to 70% guanine-cytosine residues into the primers and the absence of palindromic sequences.

Analysis of DNA by Agarose Gel Electrophoresis

The PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining. For routine analysis, minigels (7 cm × 6 cm) containing 0.8% agarose in 1 × TAE (0.04 *M* Tris acetate, 0.001 *M* EDTA) were prepared. For medium-sized gels (20 × 15 cm), 1% agarose in 1 × TAE was used. Gels were stained with ethidium bromide (0.8 μg/mL). Electrophoresis was performed at 70 V for 60 min. Then the gel was visualized on ultraviolet transilluminator at 320 nm. Gels were photographed by

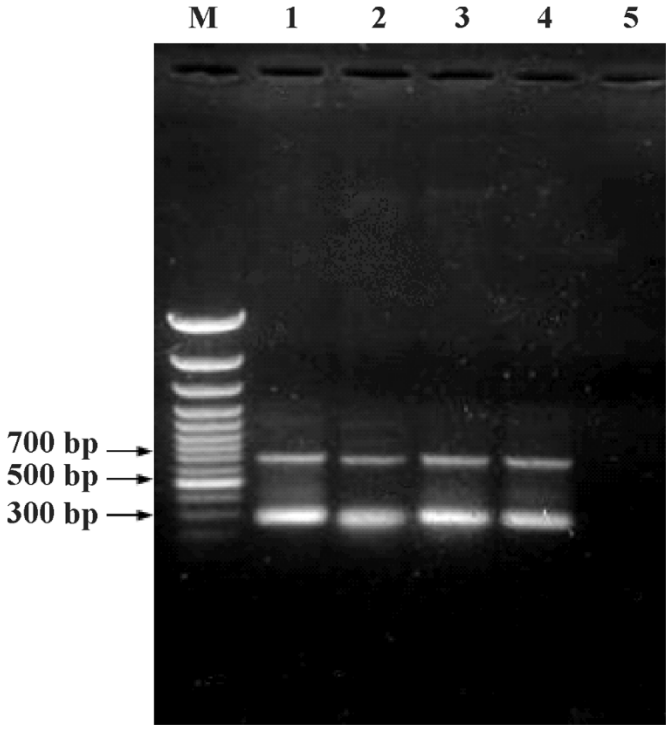

Figure 2. Amplification results of *Salmonella* Typhimurium; M = 100-bp DNA ladder plus marker; 1 = *Salmonella* Typhimurium Institute of Pasteur 2/15 [Refik Saydam National Culture Collection (RSKK) 28]; 2 = *Salmonella* Typhimurium Institute of Pasteur 2/16 (RSKK 29); 3 = *Salmonella* Typhimurium National Culture Type Collection 8391 (RSKK 02010); 4 = *Salmonella* Typhimurium RSKK 58; 5 = negative control.

Nikon Coolpix 4500 digital photograph machine (Nikon Inc., Melville, NY).

RESULTS AND DISCUSSION

The genus *Salmonella* consists of around 2,200 serotypes based on their antigens (Brenner, 1984). Due to the large number of *Salmonella* serotypes, work toward finding DNA markers for general use in detecting *Salmonella* serotypes appears to be a challenge, especially when studies are based on methods that rely on the random amplification of DNA fragments. Thus, reports on the application of RAPD analysis for finding detection markers for *Salmonella* serotypes are rather limited (Miyamoto et al., 1998). Miyamoto et al. (1998) have applied RAPD analysis for the detection of *Salmonella* spp. in foods and have designed their primer based on the N-terminal sequence of dulcitol 1-phosphate dehydrogenase. It is reported that the 23 *Salmonella* spp. tested in their work have all generated 2 fragments of 460 and 700 bp and that these fragments were unrelated to the dulcitol 1-phosphate dehydrogenase gene (Miyamoto et al., 1998). In this sense, the method is not entirely a gene-specific approach, and a single primer, as in the case of RAPD analysis, is used. Moreover, the use of a primer 24 bases long based on the sequence of a specific gene from *Salmonella* spp. is an approach that is not often used in RAPD analysis. Instead, arbitrarily designed short primers 8 to 10 bases long are used in the procedure (Williams et al., 1990). Application of RAPD analysis in studies related to population genetics and molecular taxonomy appears to be of great value in discovering slight differences in the genetic make-up of closely related organisms (Williams et al., 1990).

In our study, RAPD analysis was used to establish serotype-specific markers for *Salmonella* serotypes, with emphasis on *Salmonella* Typhimurium, due to its importance in causing the majority of *Salmonella* cases in Turkey (Yıldırmak et al., 1998). Namely, 10 different randomly designed primers 8 to 10 bases long (Table 2) were used in PCR in the presence of *Salmonella* Typhimurium DNA and DNA from other serotypes of *Salmonella* (data not shown). In our preliminary studies, promising results were obtained with some of these 10 primers. Primers 1, 2, and 10 gave the same amplification pattern with many *Salmonella* serotypes. Primers 4 and 5 did not yield any amplification products, whereas primer 8 produced 1 band of 300 bp. A unique and fairly intense amplification band of 550 bp was obtained with primer 7 for *Salmonella* Typhimurium and also some of the other *Salmonella* serotypes. Primers 6 and 9 were designed as degenerate primers with the expectation of increasing the number of bands amplified by the PCR. The results indicate that the degenerate primers indeed increase the number of amplified DNA fragments for *Salmonella* Typhimurium, namely, 5 to 6 bands are generated instead of 1 to 3. Accordingly, the degenerate primers 6 and 9 were not similarly effective in generating several amplification bands with all *Salmonella* Typhimurium. Furthermore, *Salmonella* Enteritidis displayed the same amplification pattern as *Salmonella* Typhimurium with these degenarate primers (data not shown). However, primer 3 that produced a distinguishable pattern against *Salmonella* Typhimurium in those preliminary studies was selected as an identification primer. This primer was tested for 8 *Salmonella* Typhimurium strains, which were characterized in our previous study (İçgen et al., 2002b). Although primer 3 is not a degenerate primer, the amplification pattern contained more than 1 band, the size of which varied between 300 and 1,000 bp, with 2 of these bands (300 and 700 bp) being rather intense, varying from one strain to another, and the remaining being faint bands (Figure 1). In the detection of microorganisms, it is often preferable to amplify a single and intense DNA fragment that will specify the microorganism of interest. A unique and fairly intense amplification band of 550 bp was obtained with primer 7 for *Salmonella* Typhimurium. In this respect, primer 7 was also tested against different *Salmonella* serotypes in RAPD analysis. However, the results indicate that an intense DNA fragment of 550 bp is amplified from 80% of the *Salmonella* serotypes tested. Therefore, this primer could not act as a detection primer for all *Salmonella* Typhimurium tested and nor was the primer amplification product specific to genus *Salmonella* (data not shown). On the other hand, according to the results with primer 3, the amplification pattern of *Salmonella typhimurium,* with the intense 700-bp product, was found to be unique among the 8 *Salmonella* Typhimurium isolates (Figure 1). Additionally, 4 more *Salmonella* Typhimurium strains from the

Table 3. Non-*Salmonella* serotypes used in the study and random amplified polymorphic DNA-PCR bands

Serotype name	Serotype designation or reference, or both	Number of strain	Bands	
			700 bp	300 bp
Salmonella Typhimurium	A6, A20, H28, H29, H31, H33, H49, ST4[1]	8	+	+
Salmonella Typhimurium	Numune Hospital[2]	12	+	+
Salmonella Typhimurium	Institute of Pasteur	2	+	+
Salmonella Typhimurium	NTCC[3]	1	+	+
Salmonella Typhimurium	RSKK[4]	1	−	+
Salmonella Enteritidis	H3, H12, H16, H19, H20, H21, H55, H59, E8, E9, E10[1]	11	−	+
Salmonella Agona	H38[5]	1	−	+
Salmonella Baildon	H23[5]	1	−	+
Salmonella Havana	H32, CH32, T6, T11, T12, T58[5]	6	−	+
Salmonella Kedougou	E1, E2[1, 5]	2	−	−/+
Salmonella Ouakam	A18, A24[5]	2	−	−
Salmonella Typhi	DFE, METU[6]	1	−	−
Salmonella Anatum	*Exconj, Rif*, IHR[7]	2	−	+
Salmonella Rissen	IHR[7]	1	−	−
Salmonella Bahrenfeld	IHR[7]	1	−	+
Salmonella Strasbourg	IHR[7]	1	−	+
Salmonella Orion	IHR[7]	1	−	−
Salmonella Glostrup	IHR[7]	1	−	+
Salmonella Paratyphi B	IHR[7]	1	−	−
Salmonella B	IHR[7]	1	−	−
Salmonella D	IHR[7]	1	−	−

[1]İçgen et al., 2002a.
[2]Yıldırmak et al., 1998.
[3]NTCC = National Type Culture Collection, United Kingdom.
[4]RSKK = Refik Saydam Culture Collection, Turkey.
[5]İçgen et al., 2002b.
[6]DFE, METU = Department of Food Engineering, Middle East Technical University, Turkey.
[7]IHR = Institute of Health Protection, Turkey.

Institute of Pasteur in France and National Culture Type Collection in the United Kingdom were obtained from Refik Saydam National Culture Collection in Turkey and were used in RAPD-PCR with primer 3 (Figure 2) to validate results obtained for *Salmonella* Typhimurium isolates. They also gave same amplification bands both at about 700 and 300 bp (Figure 2).

To investigate the specificity of the primer 3 amplification pattern to *Salmonella* Typhimurium, a further 12 clinical isolates along with the original strain were subjected to RAPD analysis with primer 3. These 12 clinic isolates from different individuals obtained from Numune Hospital were previously identified as *Salmonella* Typhimurium (Yıldırmak et al., 1998), yet they all displayed the same amplification pattern (Table 3). Serotype *Salmonella* Enteritidis is a very closely related serotype with *Salmonella* Typhimurium. Both are pathogenic for human and animals. In this respect, identification of serotype-specific DNA marker for *Salmonella* Typhimurium and differentiation from *Salmonella* Enteritidis are very important both for human health and the food industry. Therefore, to check and express the specificity of amplification results of *Salmonella* Typhimurium, primer 3 was applied to DNA of different *Salmonella Enteritidis isolates and other Salmonella* serotypes in RAPD-PCR (Figure 3). All isolates of serotype *Salmonella* Enteritidis (Figure 3) and some other *Salmonella* serotypes (Figure 4) given in Table 1 produced a 300-bp amplification band but not a 700-bp fragment with primer 3 under the same RAPD-PCR conditions with *Salmonella* Typhimurium in our study. *Salmonella* Typhi, *Salmonella* Paratyphi B, *Salmonella* Anatum, *Salmonella* Strasbourg, *Salmonella* Orion, *Salmonella* Rissen, *Salmonella* Quakam, *Escherichia coli,* and *Klebsiella pneumonia* in RAPD-PCR with primer 3 had given neither the 300-bp band nor the 700-bp band (Table 3, Table 4). Therefore, a 300-bp band could not be specific both for *Salmonella*

Table 4. *Salmonella* serotypes used in the study and random amplified polymorphic DNA-PCR bands

Serotype	Serotype designation or reference, or both	Number of strain	Bands	
			700 bp	300 bp
Escherichia coli	DFE, METU[1]	1	−	−
Klebsiella spp.	DFE, METU[1]	1	−	−
Pseudomonas fluorescens	IHR[2]	1	−	+
Pseudomonas aerogenes	IHR[2]	1	−	+

[1]DFE, METU = Department of Food Engineering, Middle East Technical University, Turkey.
[2]IHR = Institute of Health Protection, Turkey.

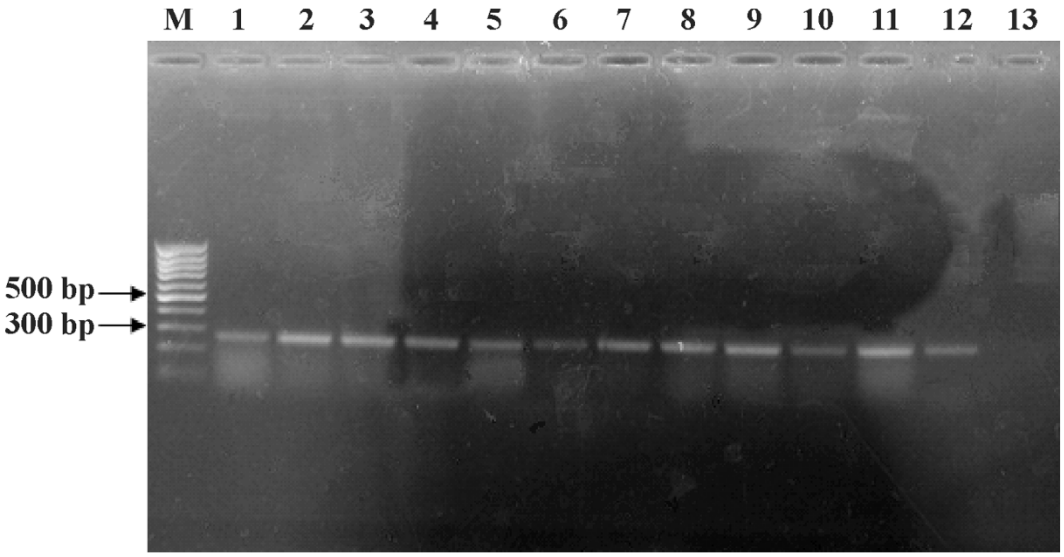

Figure 3. Amplification results of serotype *Salmonella* Enteritidis; M = 100-bp DNA ladder; 1 = *Salmonella* Enteritidis H3; 2 = *Salmonella* Enteritidis H12; 3 = *Salmonella* Enteritidis H16; 4 = *Salmonella* Enteritidis H19; 5 = *Salmonella* Enteritidis H20; 6 = *Salmonella* Enteritidis H21; 7 = *Salmonella* Enteritidis H55; 8 = *Salmonella* Enteritidis H59; 9 = *Salmonella* Enteritidis E8; 10 = *Salmonella* Enteritidis E9; 11 = *Salmonella* Enteritidis E10; 12 = positive control; 13 = negative control.

Typhimurium and for the salmonellae genus. On the other hand, specificity of the 700-bp band for *Salmonella* Typhimurium in our study was consistent with the homology comparing study results of McClelland et al. (2001), because they had discovered that there were nonhomologous regions on DNA among these bacteria. It was thought that the specific and polymorphic 700-bp region of *Salmonella* Typhimurium could be located at a region that did not show homology with closely related *Salmonella* serotypes and Enterobacteriaceae members. Moreover, 3 of the non-*Salmonella* species tested indicated absence of a similar amplification pattern (Table 4). According to the results, primer 3 generated a unique and distinguishable amplification pattern with band at about 700 bp and a band at about 300 bp with *Salmonella* Typhimurium. Thus, a 700-bp amplification band could be used as a specific DNA marker for *Salmonella* Typhimurium.

In our studies, the cell concentration between 3.5×10^9 and 8×10^9 cfu/mL gave the best results in the PCR. This is a large number for experiments that will involve direct sampling from food products. However, a preenrichment step before the PCR both lowers the concentration of inhibitory substances originating from the food material and, at the same time, reduces the risk of false-positive

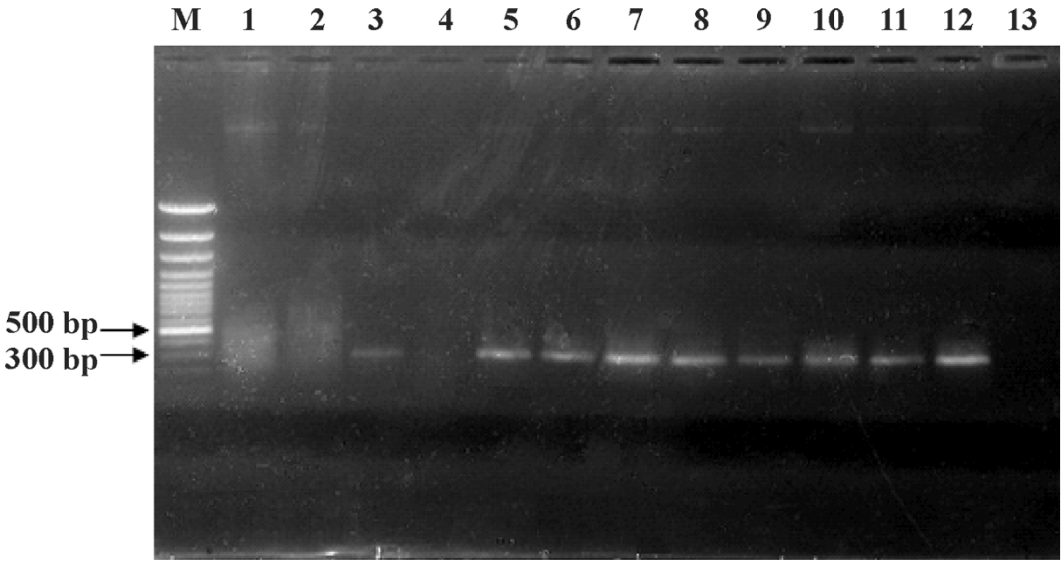

Figure 4. Amplification results of some other *Salmonella* serotypes; M = 100-bp DNA ladder plus marker; 1 = *Salmonella* Ouakam A18; 2 = *Salmonella* Ouakam A24; 3 = *Salmonella* Kedougou E1; 4 = *Salmonella* Kedougou E2; 5 = *Salmonella* Agona H38; 6 = *Salmonella* Baildon H23; 7 = *Salmonella* Havana H32; 8 = *Salmonella* Havana CH32; 9 = *Salmonella* Havana T6; 10 = *Salmonella* Havana T11; 11 = *Salmonella* Havana T12; 12 = *Salmonella* Havana T58; 13 = negative control.

results originating from the DNA of nonviable cells (Giesendorf et al., 1992; Gouws et al., 1998), likely to occur on processed food products.

The aim of this study was to develop a rapid and easy detection and identification method for *Salmonella* Typhimurium for both food industry and human health and to find a specific DNA marker for this organism. A RAPD-PCR reaction, with primer 3 with 9 bp in size, was found as an effective method. All *Salmonella* Typhimurium isolates used in this study gave strong amplification product at about 700 bp, an additional band at about 300 bp, and rarely faint bands at 800, 900, and over 1,000 bp in size. Isolates of other *Salmonella* serotypes examined (Table 1) did not produce any 700-bp band, whereas some of those gave a 300-bp amplification product. Therefore, a 300-bp amplification band has not any worth as an identification marker for serotype *Salmonella* Typhimurium or *Salmonella* genus, or both, whereas a 700-bp amplification product was found as a specific polymorphic region for *Salmonella* Typhimurium, and this band can be used as a specific marker for differentiation of *Salmonella* Typhimurium from the other serotypes.

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