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Identification of *Salmonella enterica* Serovar Typhimurium Using Specific PCR Primers Obtained by Comparative Genomics in *Salmonella* Serovars

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ABSTRACT

Salmonella enterica serovar Typhimurium is a major foodborne pathogen throughout the world. Until now, the specific target genes for the detection and identification of serovar Typhimurium have not been developed. To determine the specific probes for serovar Typhimurium, the genes of serovar Typhimurium LT2 that were expected to be unique were selected with the BLAST (Basic Local Alignment Search Tool) program within GenBank. The selected genes were compared with 11 genomic sequences of various *Salmonella* serovars by BLAST. Of these selected genes, 10 were expected to be specific to serovar Typhimurium and were not related to virulence factor genes of *Salmonella* pathogenicity island or to genes of the O and H antigens of *Salmonella.* Primers for the 10 selected genes were constructed, and PCRs were evaluated with various genomic DNAs of *Salmonella* and non-*Salmonella* strains for the specific identification of *Salmonella* serovar Typhimurium. Among all the primer sets for the 10 genes, STM4497 showed the highest degree of specificity to serovar Typhimurium. In this study, a specific primer set for *Salmonella* serovar Typhimurium was developed on the basis of the comparison of genomic sequences between *Salmonella* serovars and was validated with PCR. This method of comparative genomics to select target genes or sequences can be applied to the specific detection of microorganisms.

The *Salmonella* genus is a gram-negative bacterium and consists of two species, *Salmonella enterica* and *Salmonella bongori* (subspecies V). *Salmonella enterica* is divided into six subspecies: I, II, IIIa, IIIb, IV, and VI. *Salmonella* is classified into over 2,500 serovars on the basis of the Kauffmann-White scheme *(24, 25).* According to its antigenic profiles, *Salmonella* has different disease syndromes and host specificities. *Salmonella enterica* serovar Typhimurium has been known to cause outbreaks of salmonellosis in a wide variety of animals, including human, mouse, and chicken *(16, 20).* Serovar Typhimurium is also the most frequently isolated serovar from global foodborne outbreaks, and thus a rapid detection and identification method for this serovar is necessary in the food industry.

The serology of *Salmonella* is based on the Kauffmann-White scheme, which differentiates *Salmonella* serovars by the surface antigen differences of the somatic (O) and flagella (H) antigens *(24, 25).* This serologic method has been used to identify *Salmonella* serovars; however, this method is labor-intensive, expensive, complicated, and time-consuming. Therefore, a more rapid, simple subtyping or identification method of the *Salmonella* serovars is needed. PCR has the potential of being a powerful alternative in microbiological diagnostics because of its simplicity, rapidity, and accuracy *(23, 27, 30).* Many studies use PCR to detect *Salmonella,* and several target genes of *Salmonella* have been reported. The *invA* gene, which has mostly been used as a target gene of the *Salmonella* genus, targets but is not specific to serovar Typhimurium *(10, 13, 17, 21).* Other reported target genes, such as *ompC (14, 17), oriC (17), fimA (7), iroB (2), rfb* O antigen gene cluster *(11),* and *spaQ (29),* were specific only to the *Salmonella* genus, *Salmonella enterica,* or *Salmonella enterica* serogroup, but not specific to the serovar Typhimurium. Recently, the specific detection of *Salmonella* serovar Typhimurium by means of a multiplex PCR was reported and the target genes that were used related to the O and H antigens of serovar Typhimurium *(16).* Lim et al. *(16)* suggested *rfbJ, fliC,* and *fljB* genes, which encode for the O:4, H:i, and H:1,2 antigens, respectively, could be used to specifically target serovar Typhimurium. However, these genes were not specific and were evaluated in only a few *Salmonella* serovars. Therefore, the evaluation of specific primers with various *Salmonella* serovars is necessary for the rapid and accurate detection of serovar Typhimurium in the food industry by PCR.

Genomic sequencing projects of various *Salmonella* strains are currently under investigation or have been completed *(9).* Genomic sequencing projects for five *Salmonella* strains, *Salmonella* serovar Typhimurium LT2 *(20),* Typhi CT18 *(22),* Typhi Ty2 *(8),* Paratyphi A ATCC 9150 *(19),* and Choleraesuis SC-B67 *(5),* are completed (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/Complete.html), and other *Salmonella* strains are currently in pro-

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TABLE 1. Salmonella *serovars used in this study*

Salmonella subspecies and serovar	Serogroup	Source[a]	Strain
S. enterica subspecies I			
Typhimurium	B	ATCC 19585	LT2
		ATCC 13311	
		ATCC 14028	
Typhi	D1	ATCC 33459	
Enteritidis	D1	ATCC 4931	
Gallinarum	D1	ATCC 9184	
Pullorum		ATCC 9120	
Choleraesuis	C1	ATCC 13312	
Paratyphi C	C1	ATCC 13428	
Paratyphi B	B	ATCC 10719	
Typhimurium	B	KCPB	S9, S15, S17, S21
		BFR	G02
		FDA	DT-104
Heidelberg	B	FDA	3390H, UN-L
		BFR	G06
Agona	B	KCPB	S12, S28
		BFR	G10
Bredeney	B	BFR	G13
		FDA	1370H
Derby	B	FDA	1591H
		BFR	G14
Schwarzenground	B	KCPB	S16, S19
Brandenburg	B	BFR	G08
California	B	FDA	3515H
Saintpaul	B	BFR	G09
Enteritidis	D1	KCPB	S25, S26, S27, S29, S32, S34, S35, S38, S39, S40, S41, S53, S54, S56, S57, S63, S64, S65, S66
		BFR	G01
		FDA	3512H, H3353, Benson-1, ME-13, Me-14
Javiana	D1	FDA	2080H
Dublin	D1	BFR	G15
Haardt	C2-C3	KCPB	S30, S31, S33, S36, S37
Virginia	C2-C3	KCPB	S3, S5, S6, S7, S8
Hadar	C2-C3	KCPB	S2
		BFR	G03
Litchfield	C2-C3	BFR	G20
		FDA	3483H
Blockley	C2-C3	BFR	G11
Bovismorbificans	C2-C3	BFR	G12
Newport	C2-C3	BFR	G07
Kentucky	C2-C3	FDA	2035
Manhatan	C2-C3	FDA	1293H
Istanbul	C2-C3	KCPB	S20
Sandow	C2-C3	KCPB	S13
Infantis	C1	KCPB	S22
		BFR	G05
		FDA	1232H
Georgia	C1	KCPB	S4 S18
Montevideo	C1	BFR	G17
		FDA	1231H
Tennessee	C1	KCPB	S24
Edinburg	C1	KCPB	S10
Livingstone	C1	BFR	G16
Virchow	C1	BFR	G04
Ohio	C1	FDA	2060H

TABLE 1. *Continued*

Salmonella subspecies and serovar	Serogroup	Source[a]	Strain
Oranienburg	C1	FDA	1410H
Mbandlaka	C1	FDA	37N
Braenderup	C1	FDA	10N
Give E1	E1	FDA	1432H
Anatum	E1	FDA	1904H
Meleagridis	E1	FDA	1054H
Muenster	E1	FDA	1250H
Joal	E1	KCPB	S23
Mississippi	G	FDA	2883H
Poona	G	FDA	3417H
Paratyphi A	A	KCPB	S11
Senftenberg	E4	BFR	G19
Madelia	H	FDA	22N
Cerro	K	FDA	1325H
Illinois		FDA	2386H
Barcilly		FDA	1955H
Java B		FDA	2234H
Agona B		FDA	4000H
Mhenohen		FDA	2761H
Newington		FDA	3144H
S. enterica subspecies II			
S. enterica subsp. *salamae*		ATCC 15793	
42:r:−	T	BFR	G22
9,12:z:z39	D	BFR	G23
48:d:z6	Y	BFR	G24
42:b:e,n,x,z15	T	BFR	G25
30:l,z28:z6	N	BFR	G26
S. enterica subspecies IIIa			
S. enterica subsp. *arizonae*		ATCC 13314	
21:g,z51:−	L	BFR	G27
47:r:−	X	BFR	G28
18:z4,z32:−	K	BFR	G29
S. enterica subspecies IIIb			
S. enterica subsp. *diarizonae*		ATCC 43973	
50:z:z52	Z	BFR	G30
47:l,v:z	X	BFR	G31
18:i,v:z	K	BFR	G32
S. enterica subspecies IV			
S. enterica subsp. *houtenae*		ATCC 43974	
16:z4,z32:−	I	BFR	G33
48:g,z51:−	Y	BFR	G34
11:z4,z23:−	F	BFR	G35
S. enterica subspecies VI			
S. enterica subsp. *indica*		ATCC 43976	
45:a:e,n,x	W	BFR	G39
1,6,14,25:a:e,n,x	H	BFR	G40
41:b:1,7	S	BFR	G41
S. bongori (V)			
S. bongori		ATCC 43975	
44:r:−	V	BFR	G36
66:z65:−		BFR	G37
48:z35:−	Y	BFR	G38

[a] KCPB, Korea Consumer Protection Board *(6)*; BFR, Federal Institute for Risk Assessment *(17)*; FDA, U.S. Food and Drug Administration (CFSAN/OPDFB) *(28)*.

TABLE 2. *Non*-Salmonella *strains used in this study*

Non-*Salmonella* strain	Source	Non-*Salmonella* strain	Source
Listeria monocytogenes	ATCC 19111	*E. coli* O157:H7 932	ATCC 43894
L. monocytogenes	ATCC 19115	*Staphylococcus aureus*	ATCC 25923
L. ivanovii subsp. *Ivanovii*	ATCC 19119	*E. coli*	ATCC 27325
L. grayi	ATCC 25401	*E. coli*	ATCC 23736
L. innocua	ATCC 33090	*Citrobacter freundii*	ATCC 8090
L. welshimeri	ATCC 35897	*Shigella flexneri*	ATCC 12022
L. seeligeri	ATCC 35976	*S. sonnei*	ATCC 25931
L. monocytogenes	ATCC 19114	*Proteus vulgaris*	ATCC 29905
L. monocytogenes	ATCC 7644	*Enterococcus faecalis*	ATCC 19433
L. monocytogenes	ATCC 15313	*Rahnella aquatilis*	ATCC 15552
L. monocytogenes	ATCC 19113	*Enterobacter sakazakii*	ATCC 29544
L. monocytogenes	ATCC 19118	*Klebsiella pneumoniae* subsp. *pneumoniea*	ATCC 8724
Bacillus cereus	ATCC 14579	*Enterobacter cloacae*	ATCC 13047
B. cereus	ATCC 10876	*E. aerogenes*	ATCC 13048
Vibrio parahemolyticus	ATCC 27969	*Shigella boydii*	ATCC 8700
V. parahemolyticus	ATCC 33844		

gress. Comparative genomics in *Salmonella* biology has already been initiated by the genomic sequencing of other related *Salmonella* serovars, and this will provide a more efficient way of identifying most of the genetic differences between closely related bacteria *(9).*

In this study, specific genes of serovar Typhimurium were selected by the comparison of genomic sequences using available *Salmonella* genomic sequences. The specificity of these genes was evaluated by PCR for the identification of serovar Typhimurium without the need for serologic testing. Results suggest a new method of screening for specific genes of *Salmonella* serovar and provides a more rapid and convenient alternative for the identification of *Salmonella* serovars, enabling nonspecialized laboratories to perform these assays.

MATERIALS AND METHODS

Bacterial strains. *Salmonella* strains used in this study are listed in Table 1. Sixteen strains of *Salmonella* were purchased from the Korean Collection for Type Culture and the Korean Culture Center of Microorganisms. Forty-seven *Salmonella* strains were provided by the Korea Consumer Protection Board, and these strains belong to *Salmonella* subspecies I, including *Salmonella* serovars Hadar, Virginia, Georgia, Typhimurium, Paratyphi A, Edinburg, Agona, Sandow, Schwarzenground, Istanbul, Infantis, Joal, Tennessee, Enteritidis, and Haardt *(6).* Thirty-nine strains of *Salmonella* were provided by Dr. R. Helmuth of the Federal Institute for Risk Assessment (BFR; Molecular Biology, National *Salmonella* Reference Laboratory, Germany), which consisted of subspecies I to VI *(17).* Thirty-five *Salmonella* strains were donated by the U.S. Food and Drug Administration (CFSAN/OPDFB) *(28). Salmonella* strains were inoculated into Luria-Bertani broth medium and cultured with vigorous shaking at 37°C. Non-*Salmonella* strains as shown in Table 2 were purchased from Korean Collection for Type Culture and Korean Culture Center of Microorganisms and consisted of foodborne pathogens and Enterobacteriaceae.

Genomic DNA extraction. *Salmonella* strains from culture medium were harvested in microtubes. The genomic DNA of *Salmonella* strains were extracted with the DNeasy Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. Concentrations of extracted DNA were measured with a UV spectrophotometer (model UV-1700, Shimadzu, Tokyo, Japan). Genomic DNA of *Salmonella* strains that were within the 1.8-to-2 ratio (A_{260}/A_{280}) were used in this study.

Genomic sequences of *Salmonella* species. Table 3 lists the 12 genomic sequences of *Salmonella* strains used in this study and their sources. The genomic sequencing projects of *Salmonella* serovar Typhimurium LT2, Typhi CT18, and Typhi TY2 are completed *(8, 20, 22),* and their genomic sequences were obtained from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). Genomic sequences of *Salmonella* serovar Typhimurium DT104, Typhimurium SL1344, Enteritidis PT4, Gallinarum 287/91, and *S. bongori* 12419 were obtained from the Sanger Institute (http://www.sanger.ac.uk/Projects/Salmonella). Genomic sequences of *Salmonella* serovar Dublin and Pullorum were obtained from the University of Illinois (http://www.salmonella.org/genomics). Genomic sequences of *S. enterica* subsp. *diarizonae* and *Salmonella* serovar Paratyphi A ATCC 9150 were obtained from the Genome Sequencing Center of Washington University (http://genome.wustl.edu/project/bacterial).

Genomic sequence comparison of *Salmonella* serovar Typhimurium LT2. A total of 4,451 gene sequences (NC_003197.ffn) of *Salmonella* serovar Typhimurium LT2 were submitted to the nonredundant (nr) DNA sequence NCBI database by the BLAST (Basic Local Alignment Search Tool) program (version 2.2.5) *(1).* BLAST outputs that matched the *Salmonella* genus were eliminated and the highest scored output of each 4,451 genes was selected from BLAST outputs of each gene. On the basis of the BLAST outputs, *Salmonella*-specific expected genes that had an nr database match score of less than 40.14 and had a length less than 21 bp were compared with the genomic sequence of 11 *Salmonella* strains by BLAST (version 2.2.5). Specific genes for serovar Typhimurium were selected on the basis of BLAST outputs. The screening method for the specific genes of *Salmonella* serovar Typhimurium is shown in Figure 1.

Primer construction and PCR conditions. The oligonucleotide primers used in this study were designed by Vector NTI (Invitrogen, Carlsbad, Calif.) and listed in Table 4. Each 25-μl

TABLE 3. *Current status and international contributors of the genomic sequencing project of various* Salmonella *serovars*

Salmonella subspecies and serovar	Reference sequence	Genome size (kb)	Date	Number of coding genes	Contributor	Reference	Source
S. enterica serovar Typhimurium LT2[a]	NC_003197	4,857	Nov. 7, 2001	4,451	R. K. Wilson (GSC[b])	*20*	http://www.ncbi.nlm.nih.gov/
S. enterica serovar Typhi CT18[a]	NC_003198	4,809	Nov. 7, 2001	4,949	B. G. Barrell (Sanger Institute)	*22*	http://www.ncbi.nim.nih.gov/
S. enterica serovar Typhi Ty2[a]	NC_004631	4,791	Mar. 21, 2003	4,639	F. R. Blattner	*8*	http://www.ncbi.nim.nih.gov/
S. enterica serovar Typhimurium DT104[a]	STmDT104.dbs (NC_004513)	5,020		Finishing/gap closure	Sanger Institute		http://www.sanger.ac.uk/Projects/Salmonella/
S. enterica serovar Typhimurium SL1344[a]	STmSL1344.dbs (NC_004509)	5,091		Finishing/gap closure	Sanger Institute		http://www.sanger.ac.uk/Projects/Salmonella/
S. bongori 12419[a]	SB.dbs (NC_004548)	4,460		Finished	Sanger Institute		http://www.sanger.ac.uk/Projects/Salmonella/
S. enterica serovar Enteritidis PT4[a]	SePT4.dbs	4,686		Finishing/gap closure	Sanger Institute		http://www.sanger.ac.uk/Projects/Salmonella/
S. enterica serovar Gallinarum 287/91[a]	SG.dbs	4,869		Finishing/gap closure	Sanger Institute		http://www.sanger.ac.uk/Projects/Salmonella/
S. enterica serovar Dublin[a]	Sdu.dbs.txt (NC_002961)			Incomplete	Univ. of Illinois		http://www.salmonella.org/genomics/
S. enterica serovar Pullorum[a]	Spu.dbs.txt			Incomplete	Univ. of Illinois		http://www.salmonella.org/genomics/
S. enterica subsp. *diarizonae*[a]	Diarizonae.txt	3,600		Sequence now in shotgun	GSC		http://genome.wusti.edu/projects/bacterial/
S. enterica serovar Paratyphi A ATCC 9150[a]	SparatyphiA.txt (NC_006511)	4,585	Dec. 8, 2004	4,263	GSC	*19*	http://www.ncbi.nim.nih.gov/
S. enterica serovar Enteritidis LK5	Sen.dbs.txt (NC_002962)	4,500		Incomplete	Univ. of Illinois		http://www.salmonella.org/genomics/
S. enterica serovar Paratyphi A		5,000		Incomplete	Sanger Institute		
S. enterica subsp. *arizonae* serovar 62:z4,z23:−RSK2980		4,800		Survey shotgun complete	GSC		
S. enterica serovar Paratyphi B SPB7	NC_002963	4,800		Survey shotgun complete	GSC		
S. enterica serovar Choleraesuis str. SC-B67	NC_006905	4,755	Apr. 4, 2005	4,445	Chang Gung Genomic Medical Center	*5*	
S. enterica serovar Typhimurium TR7095	NC_003286	4,500		Incomplete	GSC		

[a] The genome sequence used in this study.
[b] GSC, Genome Sequencing Center of Washington University.

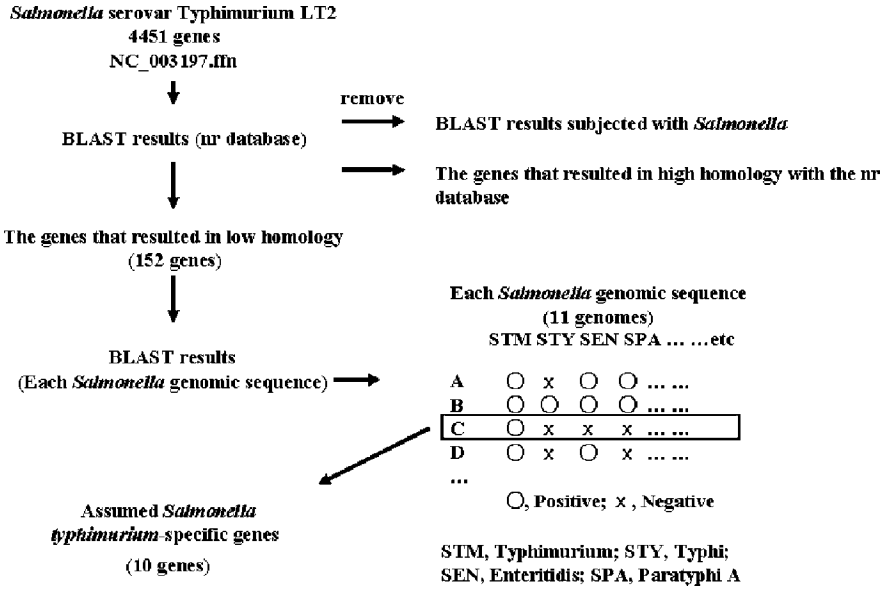

FIGURE 1. *Screening method for the specific genes of* Salmonella *serovar Typhimurium by comparing genomic sequences of various* Salmonella *serovars.*

PCR reaction contained 1× *Ex Taq* buffer (Mg^{2+} plus), 200 μmol concentration of each deoxynucleoside triphosphate, 0.5 U of *Ex Taq* DNA polymerase (TaKaRa, Otsu, Japan), 0.4 μmol of primer set, and 25 ng/μl of template DNA. PCR amplification was performed in a thermal cycler (model PC 808; Astec, Fukuoka, Japan) with an initial denaturation of 94°C for 3 min; 30 cycles of 94°C for 45 s, annealing temperature according to each primer set as listed in Table 4 for 30 s, 72°C for 30 s; and a final extension at 72°C for 3 min; reactions were kept at 4°C thereafter. Amplified products were electrophoresed on a 1.5% agarose gel in 0.5× Tris-acetate-EDTA buffer, stained with ethidium bromide, visualized under UV radiation, and photographed with a digital camera (model Coolpix 4300, Nikon, Tokyo, Japan).

RESULTS

***S. enterica* serovar Typhimurium–specific genes.** A total of 4,451 genes of *S. enterica* serovar Typhimurium LT2 (NC_003197.ffn) were submitted to the nr NCBI database by the BLASTN program (version 2.2.5). The highest scored BLAST output was selected for each gene, excluding BLAST outputs that matched the *Salmonella* genus, for a total of 4,451 BLAST outputs. Among the 4,451 genes, more than 900 genes showed low homology in the nr database, in which the matched nucleotide size is shorter than 30 bp. To minimize the number of putative *Salmonella*-specific genes, only 152 genes were selected that had an nr database match score of less than 40.14 and a length shorter than 21 bp. All 152 genes of *S. enterica* serovar Typhimurium LT2 were compared with 11 genomic sequences of varying *Salmonella* strains, including subspecies I, IIIb, and V. Ten of the 152 genes were expected only to be present in serovar Typhimurium on the basis of the size of matched-nucleotide base pairs when compared with the

TABLE 4. *Target genes of* Salmonella *serovar Typhimurium LT2 and constructed primer sets*

Target gene (synonym)	Gene size (bp)	Primer	Annealing temp (°C)	Sequence (5′–3′)	Product
STM1550	285	STM1550-f	67	AGCTA AGGGA ACGGC TTGAA	Putative cytoplasmic protein
		STM1550-r		CGTGT CATTT TGTAG ACGGC	
STM2235	1242	STM2235-f	65	TGCAG TCAGT GGCAA TAACG	Putative phage protein
		STM2235-r		CGTCA CCTTT AGCCA TCCCA	
STM2630	159	STM2630-f	65	CTGCC GCAAA TCCAT TGATG	Hypothetical protein
		STM2630-r		GTATT CAGCG CACTG CCTGG	
STM2744	216	STM2744-f	67	CCGAA AGCGG CAACG TGCGT	Putative cytoplasmic protein
		STM2744-r		CCGCA GCATC GAAGA CCACC	
STM2752	981	STM2752-f	65	TTATT CCTCC CGGTC CCGGC	Putative glucitol-specific PTS enzyme III
		STM2752-r		CCCGG CGCAG TTAAT CACCA	
STM2755	636	STM2755-f	67	AGCTG CTTTT CGACG CCGGG	Putative hexulose 6 phosphate synthase
		STM2755-r		ACCGC CAGCA TATCT GCCCC	
STM4203	360	STM4203-f	65	CTGCC TTGCA ACGTC CTGAA	Putative phage baseplate protein
		STM4203-r		CGCCA TAACA CCTCC GTTGA	
STM4214	198	STM4214-f	65	ACGCT CGCCG ACGGT CAGGA	Putative cytoplasmic protein
		STM4214-r		CTGGC ACCAG GTGAC GGCGG	
STM4497	03	STM4497-f	63	AACAA CGGCT CCGGT AATGA	Putative cytoplasmic protein
		STM4497-r3		TGACA AACTC TTGAT TCTGA	
STM4571	573	STM4571-f	65	TTTGT GCAGG CCTCA GCGGG	Putative outer membrane protein
		STM4571-r		GGGCA CTGTC ATTGG GAGCA	

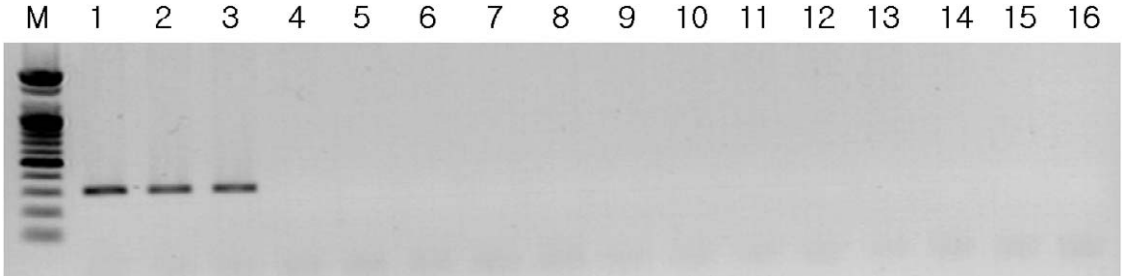

FIGURE 2. *PCR results of the STM4497-f, -r3 primer set with various* Salmonella *strains. The 310-bp DNA fragment was amplified with* Salmonella *serovar Typhimurium. M, 100-bp ladder DNA marker; lane 1, serovar Typhimurium ATCC 19585; lane 2, serovar Typhimurium ATCC 13311; lane 3, serovar Typhimurium ATCC 14028; lane 4, serovar Typhi ATCC 33459; lane 5, serovar Paratyphi B ATCC 10719; lane 6, serovar Paratyphi C ATCC 13428; lane 7, serovar Enteritidis ATCC 4931; lane 8, serovar Gallinarum ATCC 9184; lane 9, serovar Pullorum ATCC 9120; lane 10, serovar Choleraesuis ATCC 13312; lane 11,* S. salamae *ATCC 15793; lane 12,* S. arizonae *ATCC 13314; lane 13,* S. diarizonae *ATCC 43973; lane 14,* S. houtenae *ATCC 43974; lane 15,* S. indica *ATCC 43976; lane 16,* S. bongori *ATCC 43975.*

11 genomic sequences of the other *Salmonella* serovars (data not shown). The synonyms for these 10 genes are as follows: STM1550, STM2235, STM2630, STM2744, STM2752, STM2755, STM4203, STM4214, STM4497, and STM4571 *(20).* Protein functions of the selected 10 genes are listed in Table 4; most of their functions are not annotated except for the STM2752 and STM2755 genes.

The sequences of the 10 selected genes were compared again by BLASTN with the nr database (http://www.ncbi.nlm.nih.gov/BLAST) and the bacteria genome NCBI database (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). As expected, the selected 10 genes showed low homology in nucleotides with other genes based upon the nr database. The selected 10 genes showed partial similarity with the genomic DNA of *Klebsiella pneumoniae* when compared with the bacterial genomic DNA database: STM1550, 58%; STM2235, 54%; STM2630, 57%; STM4203, 58%; STM4214, 58%; STM4497, 58%; STM4571, 57%; STM2744, 93%; STM2752, 88%; and STM2755, 58%.

Accordance between the results of PCR and the comparison of genomic sequences. Primers were constructed on the basis of the sequences of the selected genes, which are provided in Table 4. PCRs were performed with genomic DNA of various *Salmonella* serovars and non-*Salmonella* bacteria. All primer sets showed amplification of the PCR products with serovar Typhimurium. PCR results were compared with results of *Salmonella* genomic sequence comparison (genome sequences of 11 *Salmonella* strains), and PCR results of seven primer sets accorded with results of *Salmonella* genomic sequences comparison against all tested serovars. The genes with different results were STM2235, STM2630, and STM2752. However, the seven primer sets (STM1550, STM2744, STM2755, STM4203, STM4214, STM4497, and STM4571 in Table 4) not only amplified PCR products with serovar Typhimurium, but also some other serovars of *Salmonella.* Primer sets of STM4203 and STM4571 amplified the expected PCR products with serovar Typhimurium and also several other serovars of *Salmonella* subspecies I. The primer set of STM1550 amplified PCR products with some serovars of subspecies I, including serovar Typhimurium, and two of five serovars of subspecies II (*S. salamae*). The STM4214 primer set amplified PCR products with some serovars of subspecies I, including serovar Typhimurium, and all three serovars of subspecies VI (*S. indica*). Results of STM2630 and STM2752 showed PCR products in subspecies IIIb and V; these results contradicted the sequence comparison results. The results of the genomic sequence comparison of the 11 *Salmonella* strains, including subspecies I, IIIb, and V, are unable to fully represent the diversity of all *Salmonella* subspecies and serovars. The primer set STM2235 amplified a PCR product with serovar Dublin, and the sequence of STM2235 showed a relatively high similarity with serovar Dublin on the basis of the results of the sequence comparison (data not shown).

Specific primer sets of *S. enterica* serovar Typhimurium. The primer sets of STM2744, STM2755, and STM4497 resulted in highly specific PCRs to *S. enterica* serovar Typhimurium among the *Salmonella* serovars. Primer sets of STM2744 and STM2755 were specific only to serovars Typhimurium and Heidelberg. In the case of primer set STM4497, a 310-bp PCR product was amplified with only serovar Typhimurium, as shown in Figure 2, which implies that this is a novel specific primer set for *S. enterica* serovar Typhimurium.

DISCUSSION

Complete genomic sequences are available for many strains of several foodborne pathogens, and comparative analysis of these sequences would be a powerful method of exploring bacterial diversity and better pathogen identification. It would also provide important insights into the evolution of bacterial virulence *(12).* In addition, comparative genomics already permits identification of novel targets for improved diagnostic and therapeutic procedures *(12).* According to previous reports, between 80% and 85% of genes are identical in both *Salmonella* serovar Typhimurium and in *Escherichia coli.* More than 2,100 *Salmonella* serovars share more than 90% DNA content *(9).* In order to remove these similar regions, we compared 4,451 genes of *Salmonella* serovar Typhimurium LT2 with the nr database provided by NCBI. The number of candidate

genes, which were expected to only be present in Typhimurium and other *Salmonella* serovars, was reduced from 4,451 to 152 genes to target genes specific to serovar Typhimurium. The 152 genes were compared with genomic sequences of other *Salmonella* serovars. Of these, 10 were expected to be specific to serovar Typhimurium.

Chan et al. *(4)* compared *Salmonella* serovar Typhimurium genes with other *Salmonella* serovars by means of microarray analysis. In this previous microarray result, the genes from STM4488 to STM4497, which encode a putative type II restriction enzyme, were only present in serovar Typhimurium, and the genes from STM4196 to STM4219, which encode a putative phage, were present only in *Salmonella* serovar Typhimurium, Paratyphi C, and Choleraesuis. In our study, STM4497 and STM4203 of the selected 10 genes are included within these regions, and resulting PCR patterns were identical with the microarray analysis as follows: PCR results of STM4497 were specific to serovar Typhimurium, and PCR results of STM4203 amplified serovar Typhimurium, Paratyphi C, and Choleraesuis (data not shown). The results of primer sets of STM2744 and STM2755 did not discriminate between serovar Typhimurium and Heidelberg. Also, previous reports suggested that Typhimurium and Heidelberg are closely related; these studies used various methods, including multilocus enzyme, nucleotide sequencing, and microarray analysis. The microarray analysis is the only analysis method that has the power to discriminate between the two serovars *(3, 15, 26).* Functions of the selected 10 genes were putatively annotated or were not annotated at all. These selected genes were not related to the virulence factor gene (*invA*) of *Salmonella* pathogenicity island *(18),* or the O and H antigenic genes (*rfbJ, fliC,* and *fljB*), which encode for the polysaccharide domain of lipopolysaccharide and the flagellin proteins in the flagella *(16).* Therefore, future studies on the functions of the selected 10 genes are necessary for additional information about *Salmonella* serovar Typhimurium.

Until now, PCR detection of *Salmonella* was limited to the *Salmonella* genus and was not specific to the serovar of Typhimurium. In addition, target genes of previous reports were specific only to the *Salmonella* genus. Lim et al. *(16)* suggested that a multiplex PCR consisting of three genes related to the O and H antigens could be used for the detection of *Salmonella* serovar Typhimurium. However, each of these three genes was not specific to the serovar Typhimurium, and only 16 of more than 2,500 *Salmonella* serovars were tested. Other studies *(2, 7, 10, 11, 13, 14, 17, 21)* reported finding specific genes that target only serovar Typhimurium and *Salmonella* spp., such as the *invA, spvC, ompC, oriC, fimA, iroB,* and *fliC* genes. We used BLAST to compare sequences of these genes to the bacterial genomic DNA database. However, the sequences of these genes were present not only in *Salmonella* serovars, but also in *E. coli, E. coli* O157:H7, *Shigella flexneri, Citrobacter freundii,* and *Klebsiella pneumoniae.*

In this study, we sought to find new target genes specific to *Salmonella* serovar Typhimurium by using the genomic sequences of *Salmonella* and proved the specificity of these selected target genes by PCR. Among the 10 candidate genes expected to be specific to serovar Typhimurium, two primer sets that targeted STM2744 and STM2755 were relatively specific, and one primer set that targeted STM4497 had the highest degree of specificity to serovar Typhimurium when compared with the various other *Salmonella* serovars. In conclusion, the PCR method used to detect serovar Typhimurium and to discriminate among various *Salmonella* serovars was successfully developed by the comparison of genomic sequences. The PCR results indicate that specific primer sets can correctly identify serovar Typhimurium, which suggests the possibility of new screening methods for specific marker genes and probes by means of comparative genomic analysis for the detection of foodborne pathogens.

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