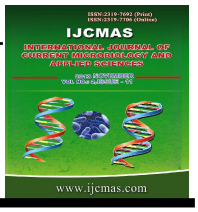
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 1-12**
http://www.ijcmas.com



Original Research Article

TaqMan Probe Real-time PCR Detection of Foodborne *Salmonella enterica* and Its Six Serovars

Jijuan Cao[1]*, Longyan Xu[2], Muyun Yuan[2], Bixia Ke[3], Dapeng Xiang[2], Changwen Ke[3], Ce Rong[3,4]

[1]Liaoning Entry-Exit Inspection and Quarantine Bureau, China
[2]Guangdong Entry-Exit Inspection and Quarantine Bureau, China
[3]Center for Disease Control and Prevention of Guangdong Province, China
[4] Dalian Polytechnic University, China
*****Corresponding author*

Keywords

Fluorescent real-time PCR; *Salmonella enteritidis; Salmonella typhimurium; Salmonella choleraesuis; Salmonella Gallinarum.*

A B S T R A C T

This study aims to establish a TaqMan probe real-time PCR method to detect *Salmonella enterica* (*S. enterica*.) and its common pathogenic serovars *S.* Enteritidis (*SE*), *S. typhimurium (STm), S. paratyphi C (SPC), S. choleraesuis (SC), S. typhi* (*ST*), and *S. gallinarum* (*SG*). TaqMan real-time PCR primers and probes were designed according to GenBank-released sequences of *S. enterica* and its serovars, and the detection methods were established simultaneously. Our results showed that the designed primers and probes were highly specific. The PCR Ct values showed a good linear relationship with the template concentrations of strains. The amplification efficiency of PCR was high, with linear coefficients (R^2) greater than 0.994. The detection sensitivity for simulated contaminated samples reached 2-5 cfu/mL. These results indicate that the method established in this study is rapid, effective, specific, and highly sensitive, which can be used in specific detection of foodborne *S. enterica* and its 6 serovars.

Introduction

S. enterica of family Enterobacteriaceae are important human enteric pathogens, which can proliferate or cause illness in gastroenteric tract of animal too. Among the currently known more than 2,500 serovars of *S. enterica*, more than 200 are found in China. There are 2 major types of diseases caused by *S. enterica*. The most common infection of *S. enterica* is acute gastroenteritis, which is resulted from the ingestion of food contaminated by a large number of *STm, SC, SE,* etc. (Chen *et al.*, 2011; Mastroeni and Maskell 2006). Another type of important diseases caused by *S. enterica* is typhoid and paratyphoid, which are a unique acute systemic febrile infection of monocytes, mainly by *ST* and *SPC,* occasionally by *STm*. Fowl typhoid is a septic infectious disease of poultry caused by *SG,* occurring mainly in chickens and turkeys. Fowl typhoid is distributed worldwide and causes serious damages, with a mortality rate of 10-50% or higher. *SE, STm, SPC, SC, ST,* and *SG*

are the most common disease-causing *S.enterica* serovars. The detection of foodborne *S. enterica* and serovars mainly relies on the traditional culture methods, which require selective enrichment culture of bacteria, biochemical identification, and serotyping, usually taking 5-6 days to obtain the test results, which is not conducive to a timely diagnosis, search for pathogen sources, and control of the disease from spreading. Therefore, to establish a rapid detection method for *S. enterica* and common pathogenic serovars, is of great significance for the detection, prevention, and control of foodborne *S. enterica*. This study has designed specific PCR primers and TaqMan probes according to the GenBank-released gene sequences of *S. enterica* and serovars, and specifically amplified *S. enterica* and serovars *SE, STm, SPC, SC, ST,* and *SG*. The specificity, sensitivity, and amplification efficiency of the introduced fluorescent PCR amplification system, as well as its compliance with the traditional methods have also been evaluated.

Materials and Methods

Test strains

A total of 166 isolates from 34 different *Salmonella* serovars, including 164 isolates of *S. enterica* (15 *SE,* 11 *STm,* 22 *SPC,* 25 *SC,* 31 *ST,* and 34 *SG*) and 2 non-enteric *Salmonella* stains *S. bongori* (ATCC43975) and *S. arizonae* (CMCC 50214), as well as other 21 non-*Salmonella* bacteria such as *Proteus* (Table 1). The above strains were all confirmed by API 20E test strips and serological tests.

The main instruments and reagents

The real-time PCR instrument ABI 7900 was from Applied Biosciences (Foster City, CA, USA); the nucleic acid extraction instrument (12GC) and reagent MagDEA DNA 200 (GC) were purchased from Precision System Science Inc. (Livermore, CA, USA); the PCR buffer, dNTP, ROX dye, and Taq DNA polymerase were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. (Dalian, China); and the buffered peptone water (BP) and tetrathionate broth (TTB) were purchased from Beijing Land Bridge Technology Co., Ltd. (Beijing, China).

Design of primers and probes

The PCR primers and Taqman probes for specific amplification of *S. enterica* and serovars *SE, STm, SPC, SC, ST,* and *SG* were designed using Oligo 6.0 software based on the gene sequences released by GenBank, and were synthesized by TaKaRa Biotechnology (Dalian) Co., Ltd. (Dalian, China). The primer and probe sequences are shown in Table 2.

DNA extraction and specificity of the test

All *Salmonella* isolates were cultured in BP at 37 ℃ for 10 h, and 10 mL of culture were then inoculated into TTB and cultivated at 44.5 ℃ for 18 h. Bacterial suspension (1 mL) was transferred into centrifuge tubes and centrifuged at 12,000 r/min for 5 min, and the supernatant was removed. The pellet was washed with 1 mL deionized water, and the supernatant was discarded after centrifugation at 12000 r/min for 3 min. The wash was repeated for 2 more tines. The pellet was finally resuspended in 200 μL deionized water and placed into nucleic acid extraction instrument for DNA extraction. *Proteus* and other non-*Salmonella* negative control strains were cultured

overnight with corresponding media. Bacteria were washed, and DNAs were extracted as described above. DNAs from all strains were used in the specificity test of *S. enterica* and 6 serovars *SE, STm, SPC, SC, ST,* and *SG*.

Real-time PCR reaction system and condition

The real-time PCR reaction was at a total volume of 30 μL, containing template DNA 1 μL, 10× TaqMan buffer 4 μL, 5 mM $MgCl_2$ 2 μL, 2.5 mML dNTPs 3 μL, TaqMan probes 20 μM 1 μL, 20 μM forward and reverse primers 1 μL each, UNG enzyme (0.55 U) 0.2 μL, Taq polymerase (2.5 U/μL) 3 μL, deionized water 13.8 μL. The condition for real-time PCR reaction was as follows: 95 ℃ 30 s; 95 ℃ 5 s, 60 ℃ 34 s, 40 cycles.

The logarithmic phase of real-time PCR amplification curve was evident, and the positive criterion was set as Ct<37; especially, amplification displaying obvious logarithmic phase at Ct<35 was directly determined as positive. Amplifications became evident at Ct values between 35 to 37 were considered suspicious, and were repeated with increased amount of template; if an evident logarithmic amplification phase appeared, the amplification was determined as positive, otherwise it was considered negative.

Simulated sample and sensitivity test

Salmonella-free chicken and fish samples determined by culture identification according to the National Food Safety Standard for Food Microbiological Examination: *Salmonella* (GB4789.4-2010) were used in the test. Chicken and fish samples (25 g each) were added with 4 different dilutions of bacterial suspensions of the *SE, STm, SPC, SC, ST,* and *SG*, respectively; sample without the addition of bacteria served as negative control. There were a total of 48 simulated samples. Each sample was then added with 225 mL BP, flapped with beater for 2 min, and cultured at 37 ℃ for 10 h. Bacterial culture (10 mL) was then inoculated into 90 mL TTB and cultured at 44.5 ℃ for 18 h. DNAs were extracted from the TTB culture and used for fluorescent real-time PCR amplification. Meanwhile, different dilutions of the simulated contaminated samples were also used in real-time PCR detection to test the assay sensitivity. All simulated samples were also identified by culture method in accordance with the National Food Safety Standard for Food Microbiological Examination: *Salmonella* (GB4789.4-2010) in parallel with the real-time PCR detection.

Result and Discussion

The results of specificity test for *S.enterica*

The DNAs extracted from 164 isolates of *S. enterica* and 21 isolates of *Proteus* and other non-*Salmonella* bacteria, as well as *S. bongori* and *S. arizonae* (Table 1) were detected by real-time PCR with Spp F/Spp R primers and Spp P probe (Table 2). As shown in Figure 1, the results of specificity test for *S. enterica* indicated that DNAs from all 164 *S. enterica* displayed positive amplification curve, whereas DNA samples from *S. bongori, S. arizonae,* and 21 non-*Salmonella* stains exhibited negative results, demonstrating that the Spp F/Spp R primers and Spp P probe designed in this study have good detection specificity.

Specificity test for SE, STm, ST, and SG

DNAs from 15 *SE*, 11 *STm*, 31 *ST*, 34 *SG*,

other *S. enterica* serovars, and 21 *Proteus* and other non-*Salmonella* strains, as well as *S. bongori* (ATCC43975) and *S. arizonae* (CMCC 50214), as listed in Table 1, were amplified by real-time PCR with primers and probes specific for *SE, STm, ST,* and *SG,* respectively (Table 2). The results showed that all *SE, STm, ST,* and *SG* DNA samples showed corresponding positive amplification curves, whereas DNA samples from other *S. enterica* serovars, 21 *Proteus* and other non-*Salmonella* strains, as well as *S. bongori* (ATCC43975) and *S. arizonae* (CMCC 50214) all displayed negative results, demonstrating that the designed respective detection primers and probes for the 4 serovars *SE, STm, ST,* and *SG* were highly specific.

Serially-diluted DNA samples from serovars *SE* (ATCC 13076), *STm* (ATCC 14028), *ST* (CMCC 50071), and *SG* (IQCC 10527) were used as templates in real-time PCR amplification. As shown in Table 3, the Ct values of respective *SE, STm, ST,* and *SG* displayed a good linear relationship with corresponding template concentration (all R^2<0.994), and the amplification efficiency was higher than 88.2%.

Specificity test for SPC and SC

As shown in Figure 2, DNAs from 22 *SPC*, 25 *SC*, other *S. enterica* serovars, and 21 *Proteus* and other non-*Salmonella* strains, as well as *S. bongori* (ATCC43975) and *S. arizonae* (CMCC 50214), which listed in Table 1, were amplified by real-time PCR with primers and probes specific for *SPC and SC* respectively (Table 2). As shown in Figure 3, DNAs extracted from all 22 *SPC* and 25 *SC* serovars showed positive amplification curve with *SC* primers and probe, whereas DNAs from other *S. enterica* serovars, 21 *Proteus* and other non-*Salmonella* stains, as well as *S. bongori* and *S. arizonae* displayed negative results. These results indicated that *SC* primers and probe in this study can specifically detect both *SPC* and *SC*.

Detection of the simulated samples and the sensitivity test

After detection with fluorescent real-time PCR, A total of 48 simulated samples added with different concentrations of respective bacteria, exhibited expected amplification results for the corresponding *SE, STm, SPC, SC, ST,* and *SG* (Table 4-6). In consistence, serovars *SE, STm, SPC, SC, ST,* and *SG* matching with the respective bacteria added were successfully isolated and identified from the simulated samples, according to the National Food Safety Standard for Food Microbiological Examination: *Salmonella* (GB4789.4-2010). It can be observed in Table 4-6 that the sensitivity of real-time PCR detection of simulated samples added with *SE, STm, SPC, SC, ST,* and *SG,* respectively, reached 2-5 cfu/mL.

The traditional method of identification of *S. enterica* requires initial pathogen isolation, subsequent biochemical identification, and serotyping; however, the cross-reactivity between different serovars interferes with the accuracy of serological diagnosis. For example, the pilus protein of *SE* is also expressed by the group D *Salmonella* serovars; the agglutination test using pilus protein often cross-reacts with *SG* as well as *S.* Pullorum (Cooper *et al.*, 1989; Hosle and Grant 1990; Zamora *et a1*., 1999).

Table.1 Information of the experimental strains

Strain name	**No.**	**Source**	**Strain name**	**No.**	**Source**
Salmonella Enteritidis	ATCC 13076	ATCC	*Salmonella* Hvittingfoss	Sa 8	Gecko
Salmonella Enteritidis	*SE*1-*SE*13	Human isolate 13	*Salmonella* Munster	Sa 10	Meat and bone meal
Salmonella Enteritidis	*SE*14	Frozen chicken	*Salmonella* Tennessee	Sa 12	Chilled fresh chicken
Salmonella Typhimurium	ATCC 14028	ATCC	*Salmonella* Aarhus	Sa 14	Red fishmeal
Salmonella Typhimurium	*STm*1-*STm*10	Isolate 10	*Salmonella* Liverpool	Sa 16	Nucleic acid yeast
Salmonella Choleraesuis	IQCC 10502	IQCC	*Salmonella* Lexington	Sa 18	Gecko
Salmonella Choleraesuis	SC1-SC24	Isolate 24	*Salmonella* Ruzizi II	Sa 20	Pig nose
Salmonella Gallinarum	CMCC 50770	CMCC	*Staphylococcus aureu*	ATCC 6538	ATCC
Salmonella Gallinarum	SG1-SG33	Isolate 33	*Escherichia coli*	ATCC 25922	ATCC
Salmonella Paratyphi A	CMCC 50002	CMCC	*Shigella* sonnei	CMCC 51334	CMCC
Salmonella Paratyphi B	CMCC 50316	CMCC	*Yersinia enterocolitica*	CMCC 52221	CMCC
Salmonella Paratyphi C	IQCC 10527	IQCC	*Klebsiella pneumoniae*	CMCC 46102	CMCC
Salmonella Paratyphi C	SP1-SP21	Isolate 21	*Hafnia alvei*	CMCC 12712	CMCC
Salmonella Typhi	CMCC 50071	CMCC	*Citrobacter freundii*	CMCC 48017	CMCC
Salmonella Typhi	ST1-ST30	Isolate 30	*Citrobacter amalonaticus*	ATCC 25405	ATCC
Salmonella Pullorum	CVCC 528	CVCC	*Pseudomon asaeruginosa*	AS 10118	Standard strain
Salmonella Enteritidis	ATCC 13076	ATCC	*proteusbacillus*	NICPBP 49005	NICPBP
Salmonella Meleagridis	CICC 21526	CICC	*marcescensSerratia*	CMCC 1.646	CMCC
Salmonella Agona	Sa 1	Frozen pig kidney	*Bacillus subtilis*	CMCC 11630	CMCC
Salmonella Derby	Sa 3	Frozen pig stomach	*Listeria monocytogenes*	ATCC 19115	ATCC
Salmonella Rissen	Sa 5	Pig stomach	*Enterobacter sakazakii*	ATCC 29544	ATCC

Salmonella Anatum	Sa 7	Feed	*Shigella* Boydit	CMCC 51582	CMCC
Salmonella Lexington	Sa 9	Mako shark cartilage	*Shigella* dysenteriae	NICPBP 51252	NICPBP
Salmonella Uppsala	Sa 11	Frozen squid	*Yersinia Pseudotuberculosis*	CMCC 53510	CMCC
Salmonella Montevideo	Sa 13	Bovine meat and bone meal	*VibrioParahemolyticus*	ATCC 33847	ATCC
Salmonella Blegdam	Sa 15	Chicken	*Bacillus cereus*	CMCC 11626	CMCC
Salmonella Istanbul	Sa 17	Frozen turkey wing	*Pseudomon asaeruginosa*	AS10120	Standard strain
Salmonella Tornow	Sa 19	Gecko	*proteusbacillus*	HB7131	Standard strain
Salmonella Dublin	CMCC 50042	CMCC	*Pseudomon asaeruginosa*	AS10124	Standard strain
Salmonella Lomita	Sa 2	Meat and bone meal	*Salmonella arizonae*	CMCC 50214	CMCC
Salmonella Orion	Sa 4	Meat and bone meal	*Salmonella bongori*	ATCC 43975	ATCC
Salmonella Senftenberg	Sa 6	Fishmeal			

Note: ATCC, American Type Culture Collection; CMCC, Chinese National Center for Medical Culture Collections; IQCC, Chinese Academy of Inspection and Quarantine; CVCC, China Veterinary Culture Collection Center; CICC, China Center of Industrial Culture Collection; and NICPBP: National Institute for the Control of Pharmaceutical and Biological Products.

Even a purified monoclonal antibody against SEFl4 pilus antigen also cross-reacts with *S.* Dublin in the latex agglutination test (Rajashekara *et a1*., 1999). Therefore, such cross-reactivity in the serum-based diagnostic methods results in certain percentage of false-positive and other issues. With the development of molecular biology techniques, there are reports of using genes *inv*A, 16s rRNA, *Spv*C, *inv*B, *fim*A, *agf*A, SEFl4, *sef*A, sdf, *ssa*Q, *fim*Y as respective targets to detect the *S. enterica* spp. with conventional or fluorescent real-time PCR (Chiu and Jonathan 1996; Dan *et al.,* 2011; Deng *et al.,* 2009; Doran *et a1*., 1993; Guo *et al.,* 2000; Kwang *et al.,* 1996; Rahn *et al.,* 1992; Thomson *et al.,* 2008; Turcotte and Woodward 1993; Wang *et al.,* 2011). However, these methods can only detect the virulence genes of *S. enterica*. but cannot determine the serotypes. Although the Bax system developed by DuPont company uses fluorescent PCR technique to detect the *S. enterica* to the genus level with high specificity, it still cannot distinguish among serovars. de Freitas *et al.,* (2010) designed primers against

Table.2 The primer and probe sequences of fluorescent real-time PCR.

Bacterial type	Primer sequences (5′-3′)	Product size (bp)	GenBank No.
Salmonella enterica (Spp)	SppF: TCACCGAAAGACCAACAGAAG SppR: GAACTCGCTGAAATGAGCAG SppP: FAM-TGGCAGGAGAAGTACCCACAGG-TAMRA	123	U43344.1
Salmonella Enteritidis (*SE*)	SEF: CTCATTCTGACCTCTAAGCCG SER: CTGGTACTTACGATGACAACTTC SEP: FAM-CTGGCGAATGGTGAGCAGACAAC-TAMRA	141	AF370707.1
Salmonella Typhimurium (*STm*)	STmF: ATTTCTGGATATGGTACTGAGGC STmR: AAATGAGTTGCTTTACGCTGC STmP: FAM-CGGGATTAATGTGCACTCCGGTTGTA-TAMRA	123	AERV01000023.1
Salmonella Paratyphi C (*SPC*)	SPF: AGTTGAAGCTGAACAGTCGC SPR: TCGCCAACAGAGACTTTGATC SPP: FAM-AGCCTCTATGGAAGTTCCGTCTCCT-TAMRA	108	CP000857
Salmonella Choleraesuis (*SC*)	SCF: GATAGGGCTGGTGTTGAAGAG SCR: GGTGCAGATAACTCCAACAGG SCP: FAM-AGCACGCTGGTTAGGTGGAATAACTC-TAMRA	98	AE017220.1
Salmonella Typhi (*ST*)	STF: GTGGCTATGCAGTGAAAATGG STR: CACCAAATTTCACAGCTCCAG STP: FAM-ACAGATGGTACTGGCGTTGCTCAAA-TAMRA	135	NC_016832.1
Salmonella Gallinarum (*SG*)	SGF: CGATATAGCTTACTGTGTCCCG SGR: TCATGCACTACCACCATAACG SGP: FAM-ACATCCCTCATATCGGCGCGAAC-TAMRA	145	HQ703462

Table.3 The amplification efficiency of fluorescent real-time PCR.

Serovars	Liner equation	amplification efficiency	R^2
SE	y=-3.40X+41.45	97.2%	0.997
STm	y=-3.05X+36.18	112%	0.997
ST	y=-3.46X+35.90	94.5%	0.994
SG	y=-3.64X+36.41	88.2%	0.999

Note: The equation presents the linear relationship between bacterial concentration (cfu/mL) and the Ct value, where y is the corresponding Ct value, and x is the logarithm of the initial concentration of the template; R^2 is the correlation coefficient between the Ct value and the concentration of bacteria.

Table.4 Fluorescent real-time PCR detection of *SE* and *STm* in simulated contaminated samples.

Sample	*SE*			*STm*			GB 4789.4-2010
	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	
Chicken	0	0	-	0	0	-	-
	4	3.0×10^6	13.62	2	3.6×10^6	26.13	+
	35	3.1×10^7	12.50	26	2.9×10^7	13.99	+
	348	2.9×10^8	10.82	310	3.3×10^8	13.78	+
Fish	0	0	-	0	0	-	-
	4	3.0×10^6	24.21	2	3.6×10^6	15.52	+
	35	3.1×10^7	22.78	26	2.9×10^7	13.04	+
	348	2.9×10^8	20.70	310	3.3×10^8	12.03	+

Table.5 Fluorescent real-time PCR detection of *SC* and *ST* in simulated contaminated samples

Sample	*SC*			*ST*			GB 4789.4-2010
	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	
Chicken	0	0	-	0	0	-	-
	2	2.8×10^6	32.47	4	2.2×10^6	23.07	+
	35	2.6×10^7	26.21	15	2.7×10^7	22.43	+
	370	3.6×10^8	25.12	220	2.9×10^8	22.57	+
Fish	0	0	-	0	0	-	-
	2	2.9×10^6	30.02	4	2.8×10^6	22.58	+
	35	3.5×10^7	28.98	15	2.8×10^7	21.08	+
	370	2.8×10^8	26.17	220	3.3×10^8	20.76	+

Table.6 Fluorescent real-time PCR detection of *SG* and *SPC* in simulated contaminated samples

Sample	*SG*			*SPC*			GB 4789.4-2010
	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	Bacteria added (cfu/mL)	Bacteria after culture (cfu/mL)	Ct value	
Chicken	0	0	-	0	0	-	-
	5	3.0×10^{6}	26.24	5	3.6×10^{6}	23.80	+
	18	3.2×10^{7}	24.69	41	2.7×10^{7}	21.85	+
	333	3.4×10^{8}	22.51	320	3.6×10^{8}	20.88	+
Fish	0	0	-	0	0	-	-
	5	3.1×10^{6}	30.82	5	2.9×10^{6}	23.67	+
	18	2.3×10^{7}	26.21	41	3.6×10^{7}	21.30	+
	333	4.2×10^{8}	19.60	320	2.8×10^{8}	20.83	+

Figure.1 The results of specific PCR amplification of *S. enterica*.

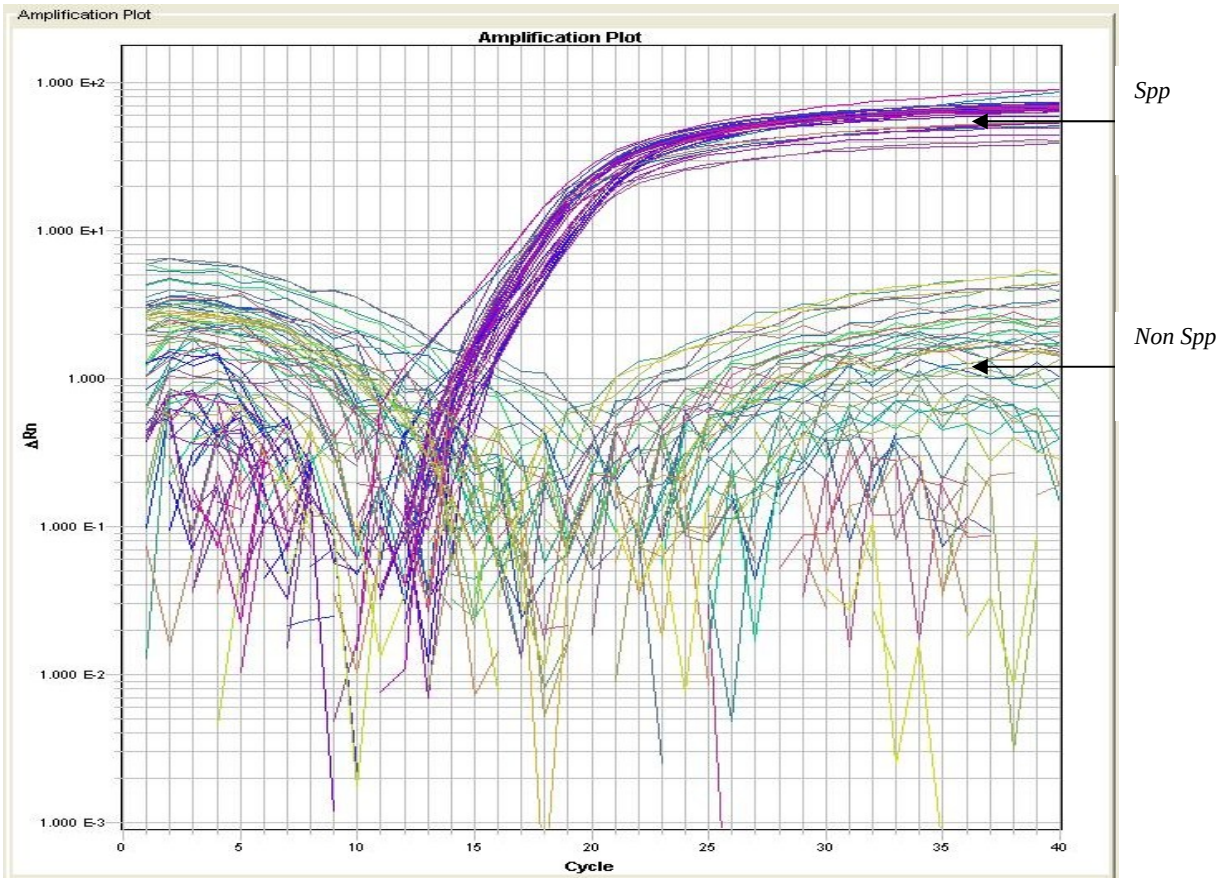


Figure.2 The results of specific PCR amplification of *SPC*.

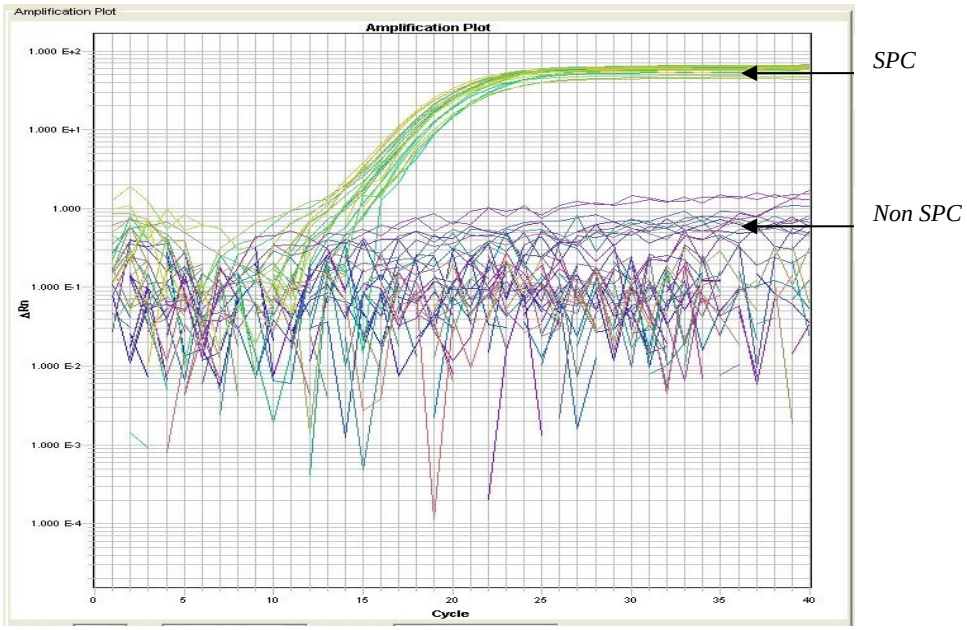
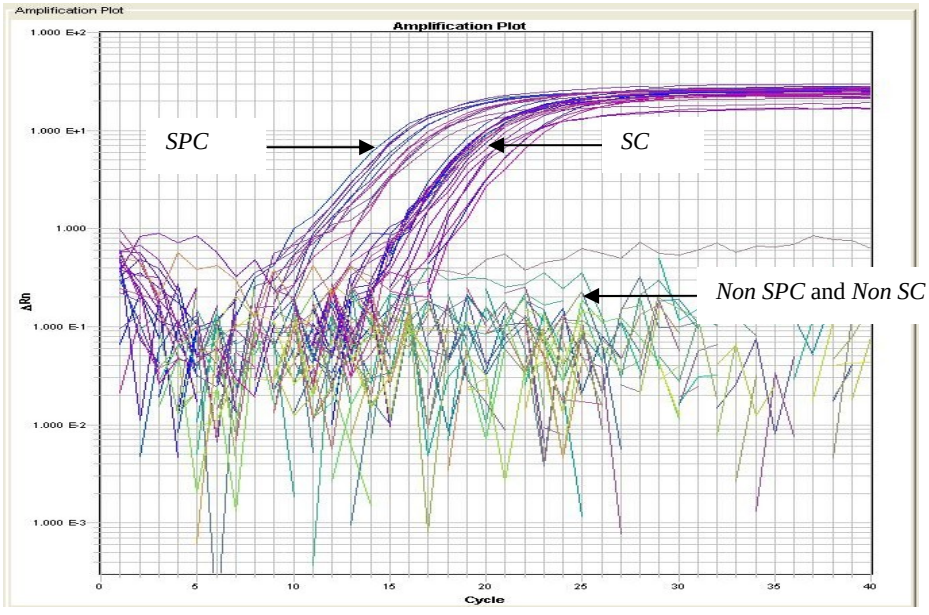


Figure.3 The results of simultaneous specific PCR amplification of *SPC* and *SC*.



*omp*C, *Sdf I*, *ViaB*, and *Spy* genes and established a multiplex PCR method to detect *S. enterica*, as well as *SE*, *ST*, and *STm*, which was then used in the detection of *S. enterica* from chicken. Kim *et al.*, (2006) also designed multiple primers based on the specific sequences of *STm* and *SPC* and established a multiplex PCR method for the detection of *STm* and *SPC;* although their method achieved good detection, it needed gel electrophoresis to determine the results, and thus the operation was complicated. O'Regan *et al.*, (2008) designed specific primers and probes against *flic*, *sefA*, *sdf*, and *acek* genes and established multiplex fluorescent PCR method for the detection of *SE*, *SG*, *STm*, *S.* Kentucky *and S.* Dublin*;* however, *sefA* as target gene was unable to accurately distinguish among *SE*, *SG*, and *S.* Dublin, while *flic* as target sequence failed to differentiate between *STm* and *S.* Kentucky. Although Woods *et al.*, (2008) also used conventional and fluorescent PCRs to detect *SC* and *SPC*, they did not evaluate the amplification efficiency of their fluorescent PCR amplification system.

This study has established a TaqMan probe-based real-time PCR method to detect foodborne *S. enterica* and its 6 serovars *SE*, *STm*, *SPC*, *SC*, *ST*, and *SG*, with good specificity. The results of real-time PCR amplification sensitivity test showed that the Ct values exhibited a good linear relationship with the concentrations of template, and the amplification efficiency of fluorescent PCR was high, with the R^2 values greater than 0.994. The detection sensitivity all reached 2-5 cfu/mL of added bacteria. The test with simulated samples verified that the detection results of the real-time PCR method established in this study are in agreement with those obtained with the conventional culture identification. Our method has the advantages of short detection time and easy operation; it can be used not only to detect the *S. enterica*. but also to differentiate among the 6 common pathogenic serovars, and thus has good application prospects.

Acknowledgement

This study was supported by the project of Natural Science Foundation of Liaoning Province (20102080) and National Science and Technology Support Program (2011BAK10B02).

References

Chen, X.L., Teng, Y., Yang, C., Li, Y., Yang, H.P., and Zhang, Z.Y. 2011. Primary Study On Rapid Detection Of Salmonella With Taqman Real-Time PCR. Modern Preven. Med. 38: 4927-4929.

Chiu, C.H., and Jonathan, T. 1996. Rapid identification of Salmonella Serovars in Feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture multiplex PCR combination assay. J.Clin. Microbiol. 34: 2619-2622.

Cooper, G.L., Nicholas, R.A., and Bracewellc, D. 1989. Serological and bacteriological investigations of chickens from flocks naturally infected with *Salmonella enteritidis*. Vet Rec. 125:567-572.

Dan, X., Liu, B., Li, X., Zhang, L., Shi, C., Zhou, M., and Shi, X. 2011. The establishment and evaluation of fluorescence quantitative PCR detection system for Salmonella with added internal standard. Acta Microbiol Sin. 51: 1119-1127.

de Freitas, C.G., Santana, A.P., da Silva, P.H., Gonçalves, V.S., Barros Mde, A., Torres, F.A., Murata, L.S., and Perecmanis, S. (2010). PCR multiplex for detection of Salmonella Enteritidis, Typhi and Typhimurium and occurrence in poultry meat. Int. J. Food. Microbiol. 139: 15-22.

Deng, S., Cheng, A., and Wang, M. 2009. Salmonella Enteritidis strain-specific PCR and rapid detection of infected ducks. Chin. J. Veter. Sci. 29: 153-157.

Doran, J.L., Collinson, S.K., Burian, J., Sarlós, G., Todd, E.C., Munro, C.K., Kay, C.M., Banser, P.A., Peterkin, P.I., Kay, W.W. 1993. DNA-based diagnostic tests for Salmonella species targeting agfA the structural gene for thin, aggregative fimbriae. J. Clin. Microbial. 31: 2263-2273.

Guo, X., Chen, J., Beuchat, L.R., and Brackett, R.E. 2000. PCR Dectection of salmonella enterica serotype Montevideo in and on Raw Tomatoes Using Primes Derived from hilA. Appl. Environ. Microbiol. 66: 5248-5252.

Hosle, B.D., and Grant, D.A. 1990. *Salmonella enteritidis* infection in pheasant chicks and poults. Vet. Rec. 126: 39-40.

Kim, S., Frye, J.G., Hu, J., Fedorka-Cray, P.J., Gautom, R., and Boyle, D.S. 2006. Multiplex PCR-based method for identification of common clinical serotypes of Salmonella enteric subsp. enterica. J. Clin. Microbiol. 44: 3608-3615.

Kwang, J., Littledike, E.T., and Keen, J.E. 1996. Use of the polymerase chain reaction for Salmonella detection. Lett. Appl. Microbiol. 22: 46-51.

Mastroeni, P., and Maskell, D. 2006. Salmonella Infections: Clinical, Immunological, and Molecular Aspects. Cambridge University Press, pp. 1.

O'Regan, E., McCabe, E., Burgess, C., McGuinness, S., Barry, T., Duffy, G., Whyte, P., and Fanning, S. 2008. Development of a real-time multiplex PCR assay for the detection of multiple Salmonella serotypes in chicken samples. BMC Microbiol. 8: 1471-2108.

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galán, J.E., Ginocchio, C., Curtiss, R. 3rd, and Gyles, C.L. 1992. Amplification of an invA gene sequence of salmonella Typhimurium by polymerase chain reaction as a specific method of detection of salmonella. Mol. Cell. Probes. 6: 271-279.

Rajashekara, G., Wanduragala, D., Halvorson, D.A., and Nagaraja, K.V. 1999. A rapid strip immunoblot assay for the specific detection of salmonella Enteritidis infection in chickens. Inter. J. Food. Microbiol. 53: 53-60.

Thomson, N.R., Clayton, D.J., Windhorst, D., Vernikos, G., Davidson, S., Churcher, C., Quail, M.A., Stevens, M., Jones, M.A., Watson, M., Barron, A., Layton, A., Pickard, D., Kingsley, R.A., Bignell, A., Clark, L., Harris, B., Ormond, D., Abdellah, Z., Brooks, K., Cherevach, I., Chillingworth, T., Woodward, J., Norberczak, H., Lord, A., Arrowsmith, C., Jagels, K., Moule, S., Mungall, K., Sanders, M., Whitehead, S., Chabalgoity, J.A., Maskell, D., Humphrey, T., Roberts, M., Barrow, P.A., Dougan, G., and Parkhill, J. 2008. Comparative genome analysis of Salmonella Enteritidis PT4 and Salmonella Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res. 18: 1624-1637.

Turcotte, C., and Woodward, M.J. 1993. Cloning, DNA nucleotide sequence and distribution of the gene encoding the SEFl4 fimbrial antigen of Salmonella Enteritidis. J. Geg. Microbiol. 139: 1477-1485.

Wang Z, Ji S, Yang Y, and Wang J. 2011. The establishment of dual fluorescence quantitative PCR detection method for Salmonella and Listeria monocytogenes. Chin. J. Food. Hyg. 23: 289-292.

Woods, D.F., Reen, F.J., Gilroy, D., Buckley, J., Frye, J.G., and Boyd, E.F. 2008. Rapid Multiplex PCR and Real-Time TaqMan PCR Assays for Detection of Salmonella enterica and the Highly Virulent Serovars Choleraesuis and Paratyphi C. J. Clini. Microbiol. 46: 4018-4022.

Zamora, B.M., Hartung, M., Hildebrandt, G., and Käsbohrer, A. 1999. Detection of antibodies to S. Enteritidis in broilers by means of indirect ELISA and Chemiluminescent Immunoassay (CLlA). Zentralbl Veterinarmed B. 46: 9-23.