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The evaluation of a PCR-based method for identification of *Salmonella enterica* serotypes from environmental samples and various food matrices

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ABSTRACT

The most commonly used method for serotyping *Salmonella* spp. is based on the Kaufmann–White scheme, and is composed of serological reactions using antibodies to LPS agglutinins. The multiplex PCR used in this investigation was established by Kim et al. to serotype the 30 most common clinical *Salmonella* serotypes, as determined by CDC. The PCR assay consists of two five-plex reactions and a single two-plex PCR reaction, based on six genetic loci from *Salmonella enterica* serotype Typhimurium and four loci from *S. enterica* serotype Typhi. In this investigation, we further evaluated the method for serotyping *Salmonella* spp. using a reference collection, environmental samples collected from a Mid-Atlantic region tomato farm study, four food matrices spiked with different *Salmonella* serotypes and a proficiency test. The PCR assay was first evaluated using DNA isolated from pure cultures of isolates obtained from various clinical and environmental samples, and then DNA isolated from broth cultures of food matrices of "Red round" and Roma tomatoes, Romaine lettuce, green onions and Serrano peppers spiked with serotypes Newport, Typhimurium, Javiana and Saintpaul, respectively. The results showed that the PCR assay correctly serotyped *Salmonella* spp. from the clinical, environmental, spiked food matrices, and proficiency test samples. These findings are significant because the PCR assay was successful in the identification of *Salmonella* in the spiked samples in a broth culture containing other non-salmonella organism. This method may be a useful resource for the food safety community.

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1. Introduction

The identification of *Salmonella* serotypes remains a highly important public health concern in the microbiological analysis of foods. The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further divided into 6 subspecies, of which *S. enterica* subspecies *enterica* serotype Enteriditis has the most clinical significance (Hadjinicolaou et al., 2009). The most common method of serotyping *Salmonella* isolates is based on the serological discrimination of O (surface polysaccharide), H (flagellar) and Vi (capsular) antigenic properties (Hadjinicolaou et al., 2009; Kim et al., 2006) and involves the recognition of both the O antigen (denotes the serogroup), and the flagellar (H) antigen. The Vi antigen is typically associated with *S. enterica* serotype Typhi. Currently, this method employs more than 150 O and H antisera for the characterization of over 2500 *Salmonella* serotypes, of which 1478 belong to the species *S. enterica* (Porwollik and McClelland, 2003). The molecular serotyping scheme used in this study is based on a modified multiplex

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PCR method reported by Kim et al. (2006), using serotypic banding patterns. The PCR method was used to serotype 30 of the most clinically relevant *Salmonella* serotypes, as reported by CDC. The method is based upon the PCR amplification of serotype-specific target genes and was selected from the analysis of previous work including whole-genome sequencing and comparative genomic hybridization studies with numerous *S. enterica* subsp. *enterica* serotypes (Kim et al., 2006; Porwollik and McClelland, 2003). The PCR method consists of two five-plex PCR reactions and one two-plex PCR reaction, and is based on six genetic loci from *S. enterica* serotype Typhimurium and four loci from *S. enterica* serotype Typhi. The remaining two loci are used to differentiate among some serotypes that have similar patterns. This method is sensitive, reproducible, and cost-effective and can easily be used in conjunction with other routine typing methods, such as pulsed-field gel electrophoresis (PFGE). In this investigation, the PCR assay by Kim et al. (Kim et al., 2006) was used to evaluate and to reestablish serotypic banding patterns, using an FDA reference collection. Then, the assay was used to serotype *Salmonella* spp. from DNA extracted from environmental isolates obtained from the Mid-Atlantic region of the Virginia Eastern Shore area. Furthermore, the lowest limit of detection was determined and the assay was used to detect and serotype *Salmonella* spp. from enrichment broth cultures of four different matrices spiked with *S. enterica* subsp. *enterica* serotypes Newport, Typhimurium, Javiana, and Saintpaul. The presence of *Salmonella* in environmental samples and each spiked food matrix was also confirmed using the culture method as described in the Bacteriological Analytical Manual (BAM), by Andrews and Hammack (2007) Chapter 5 (http://www.cfsan.fda.gov/~ebam/bam-5.html.). Lastly, the assay was used to detect and serotype four unknown proficiency test samples submitted by the Food Emergency Response Network (FERN).

2. Materials and methods

2.1. Bacterial culture conditions and DNA preparations

Cultures were prepared overnight on Trypticase Soy Agar with 5% Sheep Blood (SBA, Difco-Becton Dickinson) or in Modified Buffer Peptone Water [(mBPW), which is Buffered Peptone Water (BPW) (Difco-Becton Dickinson Co.) with an additional 3.5 g of disodium phosphate and 1.5 g of monopotassium phosphate per liter] enrichment cultures and incubated at 37 °C. Genomic DNA was isolated using the NucliSENS EasyMag instrument (Biomerieux, Inc. Hazelwood, MO) according to the manufacturer's instructions.

2.2. Validation of 30 most common serotype patterns

Studies were conducted to evaluate and confirm the serotypic banding patterns reported by Kim et al. (2006) for 30 clinically relevant serotypes of *S. enterica.* Two to three different isolates from the FDA collection of environmental and clinical isolates of *Salmonella* spp. were evaluated to confirm the 30 serotypes banding patterns. The PCR assay was also used to confirm the serotype of *S. enterica* serotypes Typhimurium, Newport, Montevideo, Seftenberg and Enteriditis, which were associated in recent outbreaks.

2.3. Molecular serotyping of salmonella isolated from tomato farm environmental study

2.3.1. Part 1. Isolating Salmonella spp.

Nine tomato farms on the Eastern Shore of Virginia's Mid-Atlantic region that use plasticulture and drip-irrigation were sampled quarterly from February 2009 to August 2010 (Micallef et al., in press). Ground water, irrigation pond water and sediment,

Table 1
Primers used in the PCR-Based Methods for serotyping *Salmonella enterica*.

		Concentration (μM)	Product Size (bp)	Anneanling Temperature (°C)	Result labels[a]
Reaction 1					
STM0716F	**STM0716R**	5	187	56	A
AACCGCTGCTTAATCCTGATGG	TGGCCCTGAGCCAGCTTTT				
STM1350F	**STM1350R**	5	171	56	B
TCAAAATTACCGGGCGCA	TTTTAAGACTACATACGCGCATGAA				
STM0839F	**STM0839R**	5	137	56	C
TCCAGTATGAAACAGGCAACGTGT	GCGACGCATTGTTCGATTGAT				
STM4525F	**STM4525R**	5	114	56	D
TGGCGGCAGAAGCGATG	CTTCATTCAGCAACTGACGCTGAG				
STM4538F	**STM4538R**	5	93	56	E
TGGTCACCGCGCGTGAT	CGAACGCCAGGTTCATTTGT				
Reaction 2					
STY0311	**STY0312**	5	301	56	F
TGGTATGGTTAAGCGGAGAATGG	GAGAGTCATAGCCCACACCAAAG				
STY0346	**STY0347**	5	262	56	G
GGCTGGAGCAGCCTTACAAAA	AAGAGTTGCCTGGCTGGTAAAA				
STY2299	**STY2300**	5	220	56	H
AATCCCCCCCCCTCAAAAA	GGTACACGTTTACTGTTTGCTGGA				
STM3845F	**STM3845R**	5	181	56	I
ATATCTCATCGTCTCCTTTTCGTGT	GAAGGTCCGGATAGGCATTCT				
STY2349F	**STY2349R**	5	124	56	J
AATTACGGAGCAGCAGATCGAGG	TGCGGCCAGCTGTTCAAAA				
Reaction 3					
PT4F	**PT4R**	5	225	56	K
GGCGATATATAAGTACGACCATCATGG	GCACGCGGCACAGTTAAAA				
STM2150F	**STM2150R**	5	101	56	L
CATAACCCGCCTCGACCTCAT	AGATGTCGTGAGAAGCGGTGG				

[a] Codes for the interpretation of the results. STM0716 (phage integrase), STM1350 (short chain acyl-CoA synthetase), STM0839 (inner membrane protein), STM4525/4524 (hsds type I restriction enzyme specificity protein), STM4538 (PTS permease), STY0311 (probable secreted protein), STY 0346/0347 (outer membrane fimbrial user protein), STY 2299/2300 (rfbH), STM3845 (inner membrane protein), STY2349 (conserved hypothetical protein), PT4 (FtsZ inhibitor protein), STM2150 (outer membrane protein).