CHAPTER 5

Molecular Detection of Foodborne Bacterial Pathogens

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INTRODUCTION

Since its discovery in the mid 1980s, the Nobel prize-winning polymerase chain reaction (PCR) has gained acceptance as a powerful microbial detection tool. The application of automated PCR technology in the medical and pharmaceutical industries has a longer history than its use in the food industry. In recent years, however, PCR technology has become more recognized for its potential at becoming a powerful alternative to cultural methods of pathogen detection in foods. The BAX PCR system manufactured by Dupont Qualicon, Inc. and the TaqMan Pathogen Detection kits by Applied Biosystems Co. are two examples of automated PCR systems that have found application in the food industry. The BAX PCR is currently an AOAC-approved PCR-based method that can be used to detect *Listeria monocytogenes*, *Listeria* species, *Salmonella* and *Escherichia coli* O157:H7 in food products, and is used by the USDA Food Safety and Inspection Service (FSIS) for detecting *L. monocytogenes* and *Salmonella* in meat, poultry, egg, and ready-to-eat meat products.

The advantage of using PCR techniques for food products is the specificity and rapidity of the tests as compared to traditional cultural techniques. However, the sensitivity of a PCR-based test for detection of pathogens in foods will depend on the type of food matrix involved. Because of the complexity of food matrices, many compounds in foods can prove to be inhibitory to PCR reactions (74). Thus, in most PCR assays, including the automated BAX and

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TaqMan systems, food samples are enriched prior to the amplification steps in order to overcome this hurdle. In addition to increasing the number of target microorganisms, and thus the detection sensitivity, enrichment is also helpful at reducing the risk of amplifying nucleic acids from dead or nonculturable cells (11). Following PCR, target species can be detected by agarose gel electrophoresis or hybridizations with labeled DNA probes (60).

Despite its limited use in the food industry, numerous studies have been reported in the literature on the development of PCR-based detection methods for foodborne pathogens. The following text will discuss the various PCR detection methods that have been successful at detecting various pathogens in different types of foods. Table 1 illustrates the target genes, primer sequences, tested foods, detection limits, specificity, and references of certain published PCR protocols.

CONVENTIONAL PCR DETECTION OF FOODBORNE PATHOGENS

Conventional PCR relies on the amplification of nucleic acids via a single pair of primers to detect one pathogen at a time, and the PCR reaction is optimized for the specific food product tested. With enrichment of tested samples, conventional PCR assays can detect *Clostridium perfringens* at 10 cfu/g in meat, milk, and salad (17), enterohemorrhagic *E. coli* O157:H7 at *ca* 10^{-1} cfu/g in beef (55), enterotoxigenic *E. coli* at 1 cfu/ml in milk (70), *L. monocytogenes* at *ca* 10^{-2} to 10^{0} cfu/g in various foods (19, 30, 48, 63), *Salmonella* at *ca* 10^{-1} to 10^{1} cfu/g in milk and meat products (11, 22, 43, 44), *Shigella* at 2 cfu/g in mayonnaise (71), *Staphylococcus aureus* at 5–15 cfu/g in skim milk and cream (65), and 10 cfu/ml in raw milk and curd (16), and *Vibrio parahaemolyticus* at 10 cfu in fish (31). However, other studies have found much higher detection limits of PCR assays for foods, without enrichment, including 4×10^{2} to 4×10^{3} cfu/g for *C. perfringens* in Korean ethnic foods (32), 10 cfu/ml for *L. monocytogenes* in milk (3), 5×10^{1} to 5×10^{2} cfu/ml for *Shigella* in various produce washes (36), 10^{2} cfu/g for *S. aureus* in skim milk and cheddar cheese (67), 3×10^{2} cfu/g for *V. parahaemolyticus* in shellfish (74), and 4×10^{4} cfu/g for *Yersinia enterocolitica* in pork (37). A PCR based on degenerate primers targeting known nonribosomal peptide synthetases (NRPS) has also been successfully developed for detecting emetic strains of *Bacillus cereus* (15).

MULTIPLEX PCR DETECTION OF FOODBORNE PATHOGENS

In multiplex PCR, two or more gene loci are simultaneously amplified in one reaction. This technique has been used widely to characterize pathogenic bacteria on the basis of their virulence factors and antigenic traits. Fratamico et al. (21) employed primers for a plasmid-encoded hemolysin gene ($hlyA_{933}$),

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
Bacillus cereus	*cerAB*	F: GAGTTAGAGAACGGTATTTATGCTGC	milk	1 cfu/ml[2, 3]	*B. cereus* group	60
		R: GCATCCCAAGTCGCTGTATGTCCAG				
	16S rRNA	F: TCGAAATTGAAAGGCGGC	NA[4]	NA	*B. cereus* group	24
		R: GGTGCCAGCTTATTCAAC				
	groEL	F: TGCAACTGTATTAGCACAAGCT	NA	NA	*B. cereus* group	9
		R: TACCACGAAGTTTGTTCACTACT				
	gyrB	F: GTTTCTGGTGGTTTACATGG R: TTTTGAGCGATTTAAATGC	coffee concentrate	10 cfu/ml[2]	*B. cereus* group	51
	NA	F: GACAAGAGAAATTTCTACGAGCAAGT R: GCAGCCTTCCAATTACTCCTTCTGCCACAGT	NA	NA	Emetic toxin producing *B. cereus*	15
Campylobacter	*flaA/B*	F: CCAAATCGGTTCAAGTTCAAATCAAAC	NA	5–20 cells[3]	*C. jejuni*,	58
		R: CCACTACCTACTGAAAATCCCGAACC			*C. coli*	
	16S *rRNA*	F1: AATCTAATGGCTTAACCATTA	NA	NA	*C. jejuni*,	46
		R1: GTAACTAGTTTAGTATTCCGG			*C. coli*	
	hippurase	F2: GAAGAGGGTTTGGGTGGTG				
		R2: AGCTAGCTTCGCATAATAACTTG				
	ask	F3: GGTATGATTTCTACAAAGCGAG				
		R3: ATAAAAGACTATCGTCGCGTG				
	16S *rRNA*	F1: ATCTAATGGCTTAACCATTAAAC	NA	NA	*C. jejuni*,	14
		R1: GGACGGTAACTAGTTTAGTATT			*C. coli*	

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
	mapA	F2: CTATTTTATTTTTGAGTGCTTGTG				
		R2: GCTTTATTTGCCATTTGTTTTATTA				
	ceuE	F3: AATTGAAAATTGCTCCAACTATG				
		R3: TGATTTTATTATTTGTAGCAGCG				
	ccoN	F: TTGGTATGGCTATAGGAACTCTTATAGCT	Raw chicken, offal, shellfish, raw meat, milk	NA	*C. jejuni*	59
		R: CACACCTGAAGTATGAAGTGGTCTAAGT				
		PROBE: TGGCATATCCTAATTTAAATTATTTACCAGGAC				
	omp50	F: TGTAAAAGCTGAACTTGCC	NA	NA	*Campylobacter*	5, 13
		R: GCCGTTCCTCTTGTCATTC				
	ccoN	F: AGAACACGCGGACCTATATA	water, milk	25 to 2 × 10^3 cfu[2]	*C. jejuni, C. coli, C. upsalensis*	27
		R: CGATGCATCCAGGAATGTAT				
Clostridium perfringens	*etxB/D*	F: TACTCATACTGTGGGAACTTCGATACAAGC	NA	NA	Type B or D strains of *C. perfringens*	23
		R: CTCATCTCCCATAACTGCACTATAATTTCC				
	pls	F1: AAGTTACCTTTGCTGCATAATCCC	cooked food, pork butchery, raw meat, milk, salad	10 cfu/g	*C. perfringens*	17

		R1: ATAGATACTCCATATCATCCTGCT				
	cpe	F2: GAAAGATCTGTATCTACAACTGCTGGTCC				
		R2: GCTGGCTAAGATTCTATATTTTTGTCCAGT				
	cpa	F1: TGCTAATGTTACTGCCGTTGATAG	NA	NA	*C. perfringens* producing different toxins	70
		R1: ATAATCCCAATCATCCCAACTATG				
	cpb	F2: AGGAGGTTTTTTTATGAAG				
		R2: TCTAAATAGCTGTTACTTTGT				
	etx	F3: TACTCATACTGTGGGAACTTCGATACAAGC				
		R3: CTCATCTCCCATAACTGCACTATAATTTCC				
	iap	F4: TTTTAACTAGTTCATTTCCTAGTTA				
		R4: TTTTTGTATTCTTTTTCTCTAGATT				
	cpe	F: ACTTAGAGTATCTATAAACTTGATACTC	Korean ethnic foods	4×10^2 cfu/g to 4.5×10^3 cfu/g[2]	Enterotoxigenic *C. perfringens*	32
		R: TAAATTGTTACTAAGCATATTATAATTAACATC				
Pathogenic Escherichia coli	*malB*	F1: TGACCACACGCTGACGCTGACCA	ground beef	10–100 cfu/g	total *E. coli*, ETEC, EIEC, EHEC	40
		R1: TTACATGACCTCGGTTTAGTTCACAGA				
	hlt	F2: TTACGGCGTTACTATCCTCTCTA				
		R2: GGTCTCGGTCAGATATGTGATTC				
	invx	F3: TCCTGCTTAGATGATGGAGGTAAT				
		R3: CTCACCATACCATCCAGAAAGAAG				
	vt	F4: TTAACCACACCCACGGCAGT				
		R4: GCTCTGGATGCATCTCTGGT				

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
Shiga-toxin-producing E. coli	*stx-2*	F1: TGTTTATGGCGGTTTTATTTG	ground beef	1 cfu/g[3, 6]	viable Shiga-toxin-producing *E. coli*	52
		R1: ATTATTAAACTGCACTTCAG				
	stx-1/2	F2: GGATCCTTTACGATAGACCTTCTCGAC				
		R2: GGATCCCACATATAAATTATTTCGCTC				
Entero-hemorrhagic E. coli (EHEC)	*stx 1*	F1: GACTGCAAAGACGTATGTAGATTCG	ground beef	1–10 cfu/g[5]	EHEC O157, O111, O26	62
		R1: ATCTATCCCTCTGACATCAACTGC				
	stx 2	F2: ATTAACCACACCCCACCG				
		R2: GTCATGGAAACCGTTGTCAC				
	$eaeA_{O26}$	F3: CTCTGCCAAAGAACTGGTTACAG				
		R3: TTTCCATGTGTATTTTCCATTGC				
	$eaeA_{O111}$	F4: GCTCCGAATTATATGATAAGAGTGG				
		R4: TCTGTGAGGATGGTAATAAATTTCC				
	$eaeA_{O157}$	F5: GTAAGTTACACTATAAAAGCACCGTCG				
		R5: TCTGTGTGGATGGTAATAAATTTTTG				
EHEC O157: H7	*stx 1*	F1: CAGTTAATGTGGTGGCGAAGG	NA	NA	EHEC O157: H7, O157:NM	8
		R1: CACCAGACAATGTAACCGCTG				
	stx 2	F2: ATCCTATTCCCGGGAGTTTACG				
		R2: GCGTCATCGTATACACAGGAGC				
	uidA	F3: GCGAAAACTGTGGAATTGGG				
		R3: TGATGCTCCATAACTTCCTG				

	eaeA	F1: CCATAATCATTTTATTTAGAGGGA	NA	NA	EHEC O157: H7, O157:NM	54
		R1: GAGAAATAAATTATATTAATAGATCGGA				
	stx 1	F2: TGTAACTGGAAAGGTGGAGTATACA				
		R2: GCTATTCTGAGTCAACGAAAAATAAC				
	stx 2	F3: GTTTTTCTTCGGTATCCTATTCC				
		R3: GATGCATCTCTGGTCATTGTATTAC				
	$fliC_{h7}$	F1: GCGCTGTCGAGTTCTATCGAGC	ground beef, blue cheese, mussels, alfalfa sprouts,	1 cfu/g	EHEC O157:H7	21
		R1: CAACGGTGACTTTATCGCCATTCC				
	stx 1	F2: TGTAACTGGAAAGGTGGAGTATACA				
		R2: GCTATTCTGAGTCAACGAAAAATAAC				
	stx 2	F3: GTTTTTCTTCGGTATCCTATTCC				
		R3: GATGCATCTCTGGTCATTGTATTAC				
	eaeA	F4: ATTACCATCCACACAGACGGT				
		R4: ACAGCGTGGTTGGATCAACCT				
	$hlyA_{O157}$	F5: ACGATGTGGTTTATTCTGGA				
		R5: CTTCACGTCACCATACATAT				
	NA	F: AGCACTGAATGACGCCGCAATTGAGACA	beef	10^{-1} cfu/g	EHEC O157: H7, O157:NM	55
		R: TCTGAGGGACCTTAATTTTCCCTGATTCTC				
Enterotoxigenic E. coli (ETEC)	*elt I*	F1: GCTGACTCTAGACCCCCAG	milk	1 cfu/ml	ETEC	69
		R1: TGTAACCATCCTCTGCCGGA				

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
	est II	F2: CTGTGTGAACATTATAGACAAATA				
		R2: ACCATTATTTGGGCGCCAAAG				
	uspA	F1: CCGATACGCTGCCAATCAGT	NA	NA	ETEC	56
		R1: ACGCAGACCGTAAGGGCCAGAT				
	elt I	F2: TATCCTCTCTATATGCACAG				
		R2: CTGTAGTGGAAGCTGTTATA				
	est I	F3: TCTTTCCCCTCTTTAGTCAG				
		R3: ACAGGCCGGATTACAACAAAG				
	est II	F4: GCCTATGCATCTACACAATC				
		R4: TGAGAAATGGACAATGTCCG				
Listeria monocytogenes	*iap*	F: CAAACTGCTAACACAGCTACT	NA	NA	*L. monocytogenes*	6
		R: GCACTTGAATTGCTGTTATTG				
	hlyA	F: CTAATCAAGACAATAAAATC	cheese	1.6×10^0 cfu/g	L. monocytogenes	19
		R: GTTAGTTCTACATCACCTGA				
	iap	F: CAAACTGCTAACACAGCTACT	cooked ground beef	3 cfu/g	viable *L. monocytogenes*	34
		R: GCACTTGAATTGCTGTTATTG				
	hlyA	F: GGGAAATCTGTCTCAGGTGATGT	cabbage	6 cfu/g[2, 5]	*L. monocytogenes*	25
		R: CGATGATTTGAACTTCATCTTTTGC				
	inlAB	F: CTTCAGGCGGATAGATTAGG	frank-furters	4×10^{-1} cfu/g	*L. monocytogenes*	30
		R: TTCGCAAGTGAGCTTACGTC				

	16S *rRNA*	F1: GCTAATACCGAATGATAAGA	fresh and ready-to-eat meat and fish, potato salads, vegetable salads, pasta, ice cream	4×10^{-2} to 2×10^{-1} cfu/g	*L. monocytogenes*	63
		F2: GGCTAATACCGAATGATGAA				
		R: AAGCAGTTACTCTTATCCT				
	actA	F: GTGATAAAATCGACGAAAATCC	soft cheeses	4×10^{-2} to 4 cfu/g	*L. monocytogenes*	48
		R: CTTGTAAAACTAGAATCTAGCG				
	hlyA	F: TTGCCAGGAATGACTAATCAAG	milk	10 cfu/ml[2,6]	*L. monocytogenes*	3
		R: ATTCACTGTAAGCCATTTCGTC				
	transcriptional regulatory gene	F: CGCAAGAAGAAATTGCCATC	NA	10 pg DNA	*L. monocytogenes*	47
		R: TCCGCGTTAGAAAAATTCCA				
Salmonella	NA	F: AGCCAACCATTGCTAAATTGGCGCA	beef, pork	NA	*Salmonella*	1, 2
		R: GGTAGAAATTCCCAGCGGGTACTG				
	oriC	F: TTATTAGGATCGCGCCAGGC	chicken	10^{-1} cfu/g[6]	*Salmonella*	20
		R: AAAGAATAACCGTTGTTCAC				
	repeat sequence	F: GATCATCCATTCGGCATTAAACA	frozen chicken	3 cfu/g	*Salmonella*	28
		R: CTCAGCGACGGAAGGGTAAATC				

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
	ompC	F: ACCGCTAACGCTCGCCTGTAT	ground beef	20 cfu/g	*Salmonella*	35
		R: AGAGGTGGACGGGTTGCTGCCGTT				
	invA	F: GCTGCGCGCGAACGGCGAAG	pork, beef, poultry meat, fermented sausage, fish	4×10^{-1} cfu/g	*Salmonella*	11
		R: TCCCGGCAGAGTTCCCATT				
	invA	F: CGGTGGTTTTAAGCGTACTCTT	ground beef, apple cider	1 cfu/g	*Salmonella*	21
		R: CGAATATGCTCCACAAGGTTA				
	16S *rRNA*	F: GTGTTGTGGTTAATAACCGCAGCA	whole milk, chicken	1 to 9 cfu/g	*Salmonella*	43
		R: TGTTBGMTCCCCACGCTTTCG				
	rfbS	F: TCACGACTTACATCCTAC	NA	10 cfu[3]	*Salmonella* serotype D	49
		R: CTGCTATATCAGCACAAC				
	sefA	F: GGCTTCGGTATCTGGTGGTGTA	egg	1.7×10^{-3} cfu/g[5]	*Salmonella* serotype D	61
		R: GGTCATTAATATTGGCCCTGAATA				
	orf6e	F: GCCGTACACGACCTTATAGA	NA	NA	*Salmonella* Enteritidis	64
		R: ACCTACAGGGGCACAATAAC				
	fliC	F: CGGTGTTGCCCAGGTTGGTAAT	NA	NA	*Salmonella* Typhimurium	64
		R: ACTGGTAAAGATGGCT				

	mdh	F: TGCCAACGGAAGTTGAAGTG	milk, chicken meat	10 cfu/g	*Salmonella* Typhimurium	44
		R: CGCATTCCACCACGCCCTTC				
Shigella	*spa*	F: AGCGATCTTACGTCTTG	carrot, celery, cauliflower, radish, broccoli, coleslaw	NA	*Shigella*	18
		R: CGAGATGTGGAGGCAT				
	rfc	F1: ATCAGGTGTCGTAATTTTA	NA	$<10^4$ cfu/g[2]	*Shigella*	26
		R1: GGGCTAAGTTCCCTC				
		F2: ATTGGTGGTGGTGGAAGATTACTGG				
		R2: TTTTGCTCCAGAAGTGAGG				
		F3: AGCTAATGCGTTTTGGGGAAT				
		R3: TCCCAATGACTGATACCATGG				
	virA	F: CTGCATTCTGGCAATCTCTTCACATC	mayonnaise	2 cfu/g	*Shigella* and EIEC	71
		R: TGATGAGCTAACTTCGTAAGCCCTCC				
	ial	F1: CTGGTAGGTATGGTGAGG	lettuce, shrimps, milk, blue cheese	10 cfu/g[7]	*Shigella* and EIEC	45
		R1: CCAGGCCAACAATTATTTCC				
		F2: TTTTTAATTAAGAGTGGGGTTTGA				
		R2: GAACCTATGTCTACCTTACCAGAAGT				

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
	ipaH	F: GTTCCTTGACCGCCTTTCCGATACCGTC	cilantro, tomato, beef, lettuce, alfalfa sprouts, apple cider, bean sprouts	50–500 cfu/ml[2]	*Shigella* and EIEC	36
		R: GCCGGTCAGCCACCCTCTGAGAGTAC				
Staphylococcus aureus	*sea*	F1: CCTTTGGAAACGGTTAAAACG	NA	100 pg DNA[3]	*S. aureus* carrying enterotoxin A to E genes	4
		R1: TCTGAACCTTCCCATCAAAAAC				
	seb	F2: TCGCATCAAACTGACAAACG				
		R2: GCAGGTACTCTATAAGTGCCTGC				
	sec	F3: CTCAAGAACTAGACATAAAAGCTAGG				
		R3: TCAAAATCGGATTAACATTATCC				
	sed	F4: CTAGTTTGGTAATATCTCCTTTAAACG				
		R4: TTAATGCTATATCTTATAGGGTAAACATC				
	see	F5: CAGTACCTATAGATAAAGTTAAAACAAGC				
		R5: TAACTTACCGTGGACCCTTC				
	tst	F1: AAGCCCTTTGTTGCTTGCG	NA	100 pg DNA[3]	*S. aureus* carrying exfoliative toxin A and B genes and the toxic shock syndrome toxin 1 gene	4

		R1: ATCGAACTTTGGCCCATACTTT				
	eta	F2: CTAGTGCATTTGTTATTCAAGACG				
		R2: TGCATTGACACCATAGTACTTATTC				
	etb	F3: ACGGCTATATACATTCAATTCAATG				
		R3: AAAGTTATTCATTTAATGCACTGTCTC				
	23S *rRNA*	F1: ACGGAGTTACAAAGGACGAC	skim milk, cream	5–15 cfu/g[7]	*S. aureus*	65
		R1: AGCTCAGCCTTAACGAGTAC				
		F2: ATCATCTGGAAAGATGAATCAA				
		R2: ATCGATTAAAACGATTATAGGT				
	entC	F1: ACACCCAACGTATTAGCAGAGAGC	skim milk, cheddar cheese	100 cfu/g[2]	enterotoxigenic *S. aureus*	67
		R1: CCTGGTGCAGGCATCATATCATAC				
	nuc	F2: AGTATATAGTGCAACTTCAACTAA				
		R2: ATCAGCGTTGTCTTCGCTCCAAAT				
	seg	F1: GCTATCGACACACTACAACC	NA	10^2 cfu/ml for pure culture	*S. aureus* carrying enterotoxin G to I genes	10
		R1: CCAAGTGATTGTCTATTGTCG				
	seh	F2: CACATCATATGCGAAAGC				
		R2: CGAATGAGTAATCTCTAGG				
	sei	F3: GATACTGGAACAGGACAAGC				
		R3: CTTACAGGCAGTCCATCTCC				
pathogenic Vibrio species	*tdh*	F: TTTCATGATTATTCAGTTT	fish	10 cfu	*V. parahaemolyticus*	31
		R: TTTGTTGGATATACACAT				

Continued

Table 5.1. Representative PCR methods for common foodborne bacterial pathogens—*cont'd*

Bacterial Pathogen	*Target Gene*	*Primer sequence (5′ to 3′)*	*Tested Food*	*Detection Limit*[1]	*Specificity*	*Reference*
	vvhA	F1: GACTATCGCATCAACAACCG	seafood	10^1–10^2 cfu/g[2, 7]	*V. vulnificus*	39
		R1: AGGTAGCGAGTATTACTGCC				
		F2: GCTATTTCACCGCCGCTCAC				
		R2: CCGCAGAGCCGTAAACCGAA				
	toxR	F: GTCTTCTGACGCAATCGTTG	NA	NA	*V. para-haemolyticus*	33
		R: ATACGAGTGGTTGCTGTCATG				
	hlyA	F: TGCGTTAAACACGAAGCGAT	oyster	6–8 cfu/g[2, 5]	*V. cholerae*	50
		R: AAGTCTTACATTGTGCTTGGGTCA				
	vvhA	F: TGTTTATGGTGAGAACGGTGACA	oyster	10^3 cfu/g[2, 5]	*V. vulnificus*	7
		R: TTCTTTATCTAGGCCCCAAACTTG				
	ORF8	F: GTTCGCATACAGTTGAGG	shellfish	3×10^2 cfu/g[2]	*V. para-haemolyticus* O3:K6	74
		R: AAGTACAGCAGGAGTGAG				
	elastase gene	F: AAACTCAGGTCTGATATACAGC	NA	25 cfu for pure culture	*V. vulnificus*	38
		R: AAGTTGCTACCTGGCGTGTTG				
	tdh	F1: CATCTTCGTACGGTTTTCTTTTTACA	mussel	NA	*V. para-haemolyticus*	12
		R1: TCTGTCCCTTTTCCTGCCC				
	trh	F2: GCCAAGTGTAACGTATTTGGATGA				
		R2: TGCCCATTTCCGCTCTCA				
	tlh	F3: CGAGAACGCAGACATTACGTTC				
		R3: TGCTCCAGATCGTGTGGTTG				

	vvh	F: TTCCAACTTCAAACCGAACTATGA	oyster	1 cfu/g[5]	*V. vulnificus*	57
		R: ATTCCAGTCGATGCGAATACGTTG				
Yersinia enterocolitica	16S *rRNA*	F1: GGAATTTAGCAGAGATGCTTTA	pork	4×10^4 cfu/g[2]	*Y. enterocolitica*	37
		R1: GGACTACGACAGACTTTATCT				
	yadA	F2: TGTTCTCATCTCCATATGCATT				
		R2: TTCTTTCTTTAATTGCGCGACA				
	ail	F: GGTCATGGTGATGTTGATTACTATTCA	ground pork	≤1 cfu/g[5]	*Y. enterocolitica*	29
		R: CGGCCCCCAGTAATACCATA				
	yst	F: AATGCTGTCTTCATTTGGAGC	ground pork, tofu	10^3 cfu/g[2, 5]	*Y. enterocolitica*	72
		R: ATCCCAATCACTACTGACTTC				

[1] Samples were enriched prior to the PCR assay to determine the detection limit, unless otherwise specified.
[2] Samples were not enriched prior to the PCR assay to determine the detection limit.
[3] Hybridization was performed to identify the PCR products.
[4] Not available.
[5] In the format of fluorogenic real-time PCR.
[6] Samples were processed via immunomagnetic separation.
[7] In the format of nested PCR

chromosomal flagella ($fliC_{H7}$; flagellar structural gene of H7 serotype), Shiga toxins (stx_1, stx_2), and attaching and effacing (*eaeA*) genes for specific identification of *E. coli* O157:H7. Similar protocols were reported for concurrent determination of multiple toxin genes in *C. perfringens* (70) and *S. aureus* (4, 10). Further, species or serotype differentiation can also be achieved via multiplex PCR. Denis et al. (14) selected 16S *rRNA*, *mapA*, and *ceuE* as the target genes for simultaneous detection of *Campylobacter jejuni* and *Campylobacter coli*. A similar assay was established for simultaneous identification of *Salmonella* sp., *S.* Enteritidis, and *S.* Typhimurium in one reaction (64). In multiplex PCR, bacterial pathogens belonging to different genera can also be screened in the same amplification system. Li and Mustapha (41) and Li et al. (42) established a multiplex PCR for simultaneous detection of *E. coli* O157:H7, *Salmonella*, and *Shigella* in apple cider, produce and raw and ready-to-eat meat products. In most situations of multiplex PCR, the optimal conditions for different primer sets may be unique and interference among different primer pairs may occur, resulting in uneven amplification of different target sequences and limited sensitivity (68). Thus, adjusting the concentration of Taq DNA polymerase, $MgCl^2$, or dNTPs, as well as the concentration ratio among the different primer pairs is needed in the optimization of the amplification system. Although to design a robust multiplex PCR assay for foods can be challenging, once optimized for the specific pathogens and food products, this method has the advantage of being cost effective and highly efficient. Because of its selectivity, sensitivity, and efficiency, a multiplex PCR protocol is very applicable and suitable for comprehensive testings of specific foods.

REVERSE TRANSCRIPTION-PCR DETECTION OF FOODBORNE PATHOGENS

The use of reverse transcription PCR (RT-PCR) in foods is limited due to the difficulty of extracting undegraded mRNA from pathogens in complex food matrices. By amplifying the *iap* mRNA, a RT-PCR was successfully developed for detecting viable *L. monocytogenes* cells in cooked ground beef, artificially contaminated with ca. 3 cfu/g, following a 2-h enrichment step (34). McIngvale et al. (52) established a similar protocol for Shiga-toxin-producing *E. coli* with optimal growth medium, incubation temperature, and aeration. The assay was validated in artificially contaminated ground beef. Viable *E. coli* O157:H7 at an initial inoculum of 1 cfu/g was detectable in the meat after a 12-h enrichment. In addition, a RT-PCR was developed for detecting mRNA from the *sefA* gene of *S.* Enteritidis (66). The sensitivity of the assay depended on the physiological state of the cells under different temperatures and pH. With the RT-PCR, it was possible to detect 10 cells of *S.* Enteritidis PT4 in contaminated minced beef and whole egg samples following a 16-h enrichment step. Although not cost-effective for routine testings of pathogens in

foods, these reports highlight the potential of RT-PCR for the detection of viable bacterial pathogens in foods.

REAL-TIME PCR DETECTION OF FOODBORNE PATHOGENS

Recent advances in fluorescent chemistries and detection instruments allow further development of PCR technology as a more efficient and sensitive tool for "real-time" microbiological analysis of foods. The use of nonspecific fluorescent double-stranded DNA-binding dyes (such as SYBRGreen or SYBRGold), or specific fluorescence resonance energy transfer technology (such as 5′-nuclease assay [TaqMan], or molecular beacon) has resulted in PCR assays with quantitative capability in a real-time manner (53). A number of real-time PCR assays have been described for the detection and quantification of *C. jejuni* (59), enterohemorrhagic *E. coli* serotypes O157, O111, and O26 in ground beef (62), *L. monocytogenes* in cabbage (25), *Salmonella* serotype D in egg (61), pathogenic *Vibrio* species in oyster (7, 50, 57), and *Y. enterocolitica* in ground pork (29, 72). Further, a sensitive multiplex real-time PCR has been developed for the simultaneous detection of *E. coli* O157:H7, *Salmonella,* and *Shigella* in pure culture and in ground beef (A. Mustapha, unpublished data). In addition to maintaining all the advantages of conventional PCR, real-time PCR has added speed and sensitivity. This technique can quantify a target DNA with greater reproducibility, which is very valuable in the quantitative assessment of microbial risks and the execution of HACCP programs in the food industry. The current drawback for using real-time PCR for routine food testing is the cost involved, not only in the equipment but the reagents.

CONCLUSIONS

The PCR has come a long way since its discovery, evolving from a tool used mainly in forensic, medical, pharmaceutical, and plant sciences to food science and the food industry. It may be one of the most remarkable discoveries of the 20th century and has opened new doors in a wide array of fields that would never have been possible prior to its utilization. Although research have shown that PCR can be a powerful method for detection of foodborne pathogens in pure culture as well as in certain foods, much more work needs to be done to truly make it the best alternative detection technique to conventional cultural methods. Until the enrichment steps can be eliminated, the rapidity of PCR assays can still be argued. Foods also are so different in their composition, resulting in a multitude of compounds that may be inhibitory to the detection of some pathogens while not affecting others, thus making it more challenging to design a one-size-fits-all PCR assay for foods.

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