AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Development of rapid molecular detection methods for foodborne pathogens in fresh produce cultivated in Oregon

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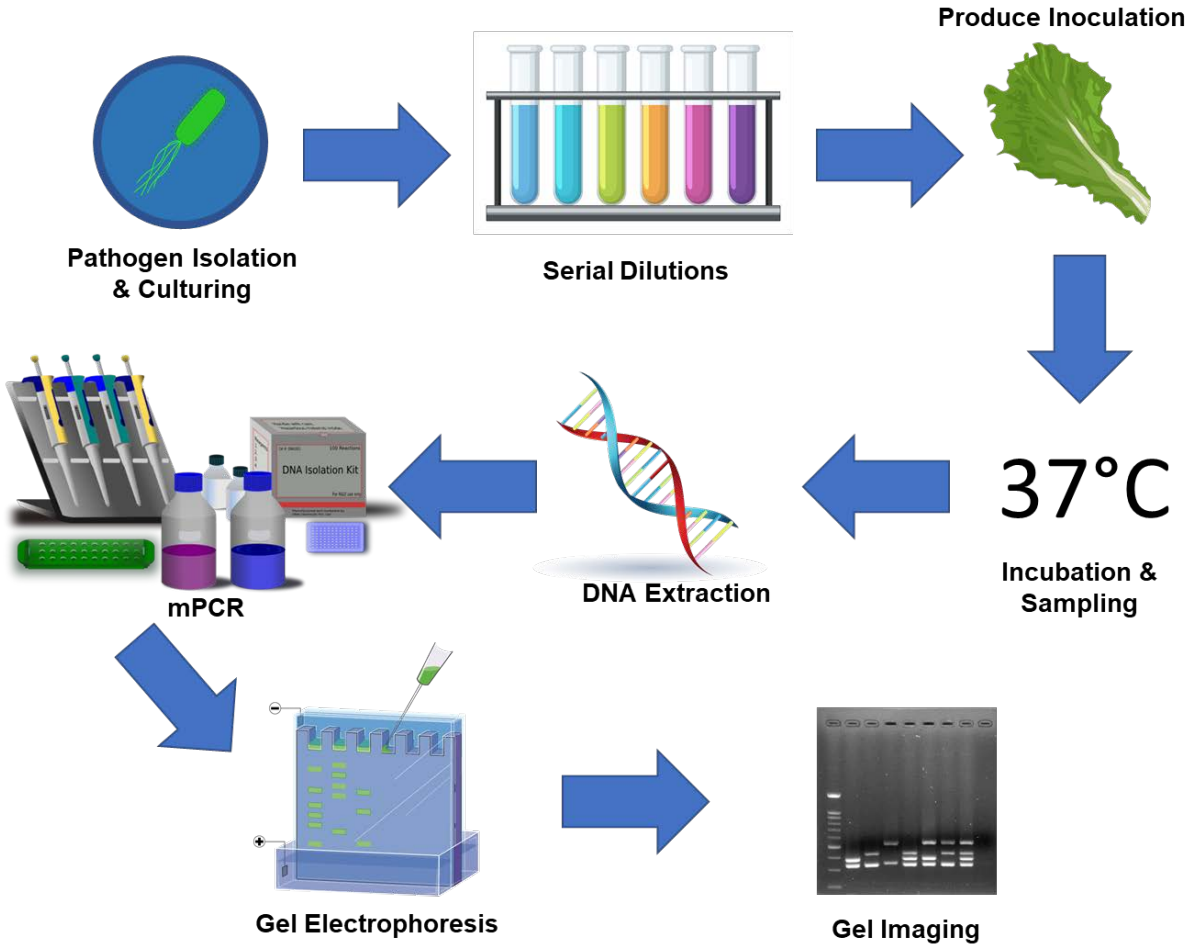
EXECUTIVE SUMMARY: Foodborne illnesses continue to be a serious concern as a public health issue for the food industry with bacterial agents being responsible for most of the hospitalizations (63.9%) and deaths (63.7%). With growing public concerns in relation to recent outbreaks associated with fresh produce, pre- and post-harvest controls of foodborne pathogens are critical to prevent further dissemination from farm to fork phase. The most notable foodborne pathogens identified in fresh produce are *Escherichia coli*, *Salmonella* subspecies I, and *Listeria monocytogenes*. The objective was to develop a multiplex PCR assay to detect multiple potential foodborne pathogens simultaneously and return results within hours versus days. *Escherichia coli* O26:H11, *Salmonella* Typhimurium LT2, and *Listeria monocytogenes* ScottA were cultivated upon respective media at 37 °C for 24 h. Single colony of each strain was selected and placed into 5 ml of broth media, followed by incubation with a 200 rpm orbital shaker at 37 °C for 18 h. Grown cultures were serially diluted to 10^{-8} and 1 ml portions (each pathogen separately + all three together) were added to stomacher bags contained 25 g of kale or romaine lettuce. A 225 ml of buffered peptone water was added to the bags and homogenized with a stomacher, followed by incubation at 37 °C. One ml aliquots were drawn in triplicates at 0, 2, 4, 6, 8, and 18 h incubation. Samples were centrifuged before genomic DNA isolation via a boiling method. The multiplex PCR assay was optimized, and the amplicons were confirmed by agarose gel (3%) electrophoresis. The developed multiplex PCR assay was able to accurately detect the presence of all three pathogens, together and separately throughout the incubation time. To date, many detection methods are available for these pathogens, but often require pre-enrichment, which results in a 5 to 7-day turnaround for results. This multiplex PCR assay allows to produce similar results within a few hours.

OBJECTIVES: The objects of this proposal are 1) **Development of the multiplex PCR assay** for simultaneous detection of 3 foodborne pathogens (*Escherichia coli*, *Salmonella* subspecies I, and *Listeria monocytogenes*) in fresh produce; 2) **Evaluation of the detection limit of the multiplex PCR assay in fresh produce (kale and romaine lettuce)**. Completion of this project using a fresh produce will be part of a comprehensive effort on our part to develop reliable and rapid detection methods.

PROCEDURES:

1. Overall project procedure



2. Bacterial growth

Listeria monocytogenes ScottA, *Salmonella* Typhimurium LT2, and *Escherichia coli* O26:H11 USDA-O26-1 (Human isolates) were cultured on Brain Heart Infusion (BHI) agar, Trypticase Soy Agar (TSA), and MacConkey agar (MAC), respectively. Frozen stock of each strain was streaked on the respective medium and a negative control plate were used to ensure no contamination was present. All plates were incubated at 37 °C for 24 h and single colony of each strain was transferred to 5 ml tubes of respective broth (in triplicate + a negative control for each). These inoculated tubes of broth were then placed into an orbital shaker and incubated at 37 °C, 200 rpm, for 24 h.

3. DNA extraction

Genomic DNA (gDNA) extraction from each grown bacterial culture was performed using a Qiagen DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Cells were grown in their respective media to an estimated 2×10^9 CFU/ml and 1 ml of each was transferred to a 1.5 ml microcentrifuge tube before being centrifuged at 13,000 rpm for 2 min in a Centrifuge 5424 R (Eppendorf, Hamburg, Germany). The supernatant was discarded, and another 1 ml of cultured broth was added to each tube before being centrifuged at 13,000 rpm for another 2 min. The supernatant was discarded again and the pellets were resuspended in 180 µl of the provided lysis buffer ATL. The tubes were then placed into a water bath at 37 °C for 30 min. A 25 µl of Proteinase K solution was added to each tube along with 200 µl of AL buffer and mixed thoroughly by vortexing with a Vortex Genie® 2 (Scientific Industries Inc, Bohemia, New York, USA). Tubes were then placed into a VWR Digital Heating Block (VWR International, Radnor, PA, USA) at 56 °C for 1 h. A 200 µl of 100 % ethanol was added to each tube followed by vortexing. Using a 1 ml pipette, the solution of each tube was transferred to respective 2 ml collection filter microcentrifuge tubes. Tubes were centrifuged at 8,000 rpm for 1 min, and then collection tubes were discarded and the columns were transferred to fresh collection tubes. 500 µl of buffer AW1 was added on top of each column and centrifuged at 8,000 rpm for 1 min. Flow through was discarded, and the columns were transferred to a fresh collection tube. A 500 µl of buffer AW2 was added to the top of each column and centrifuged at 14,000 rpm for 3 min. Flow through was discarded and columns were transferred to fresh 1.5 ml collection tubes. A 50 µl of water was added to the top of each column. Tubes were allowed to stand for 2 min at room temperature before being centrifuged at 8,000 rpm for 2 min. Flow through collected from this step was concentrated gDNA. The gDNA was analyzed using an Invitrogen Qubit 4 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to determine the concentrations and diluted to 10 ng/µl and stored in a -20 °C freezer.

4. Single PCR mixture

A variety of specific primer pairs were tested, and then through this testing, narrowed down to primer pairs, which showed a high specificity and viability for each *L*. *monocytogenes*, *S*. Typhimurium and *E*. *coli* O26:H11. The selected primer pairs for the PCR assay are listed below (Table 1). All primer pairs were synthesized by Integrated DNA Technologies (IDT, Coraville, IA, USA). The single PCR assay to evaluate the specificity of primer pairs were performed using a Nexus Gradient Mastercycler (Eppendorf, Hamburg, Germany). A total volume of 25 µl of PCR assay was composed of 12.5 µl of Takara Premix Ex *Taq*™ (Takara™, Fisher Scientific, Pittsburgh, PA, USA), 1 µl of both 16S primers (10 pmol), 1 µl of each respective primer for the individual reactions (10 pmol for eaeAF/eaeAR, 10 pmol for STM3098-f2/STM3098-r2, and 10 pmol for lmo1030-f/lmo1030-r), 1 µl of DNA template, and DNase-RNase free water to volume.

Table 1. Single PCR mixture for the evaluation of primer pair specificity.

Component	Volume	Concentration
gDNA*	1 µl	10 ng
16SFa	1 µl**	10 pmol
16SR	1 µl**	10 pmol
eaeAF	1 µl**	10 pmol
eaeAR	1 µl**	10 pmol
STM3098-f2	1 µl**	10 pmol
STM3086-r2	1 µl**	10 pmol
lmo1030-f	1 µl**	10 pmol
lmo1030-r	1 µl**	10 pmol
Ex-*Taq* Polymerase	12.5 µl	
DNase-RNase Free H_2O	9.5 µl	
Total	**25 µl**	

***** gDNA represents the 1 µl of each strain
****** For single PCR assay, only one of primer pairs was chosen

Table 2. Primer pair information utilized in this study

Species	Primer	Sequence (5'-3')	Amplicon Size (bp)	Target Gene	Reference
E. coli O26	16SFa	GCTCAGATTGAACGCTGG	320	16S	Harris et al. (2003)
	16SR	TACTGCTGCCTCCCGTA			
	eaeAF	GACCCGGCACAAGCATAAGC	384	*eaeA*	Paton et al. (1998)
	eaeAR	CCACCTGCAGCAACAAGAGG			
S. Typhimurium	STM3098-f2	TTTGGCGGCGCAGGCGATTC	423	*STM3098*	Kim et al. (2006)
	STM3098-r2	GCCTCCGCCTCATCAATCCG			
L. monocytogenes	lmo1030-F	GCTTGTATTCACTTGGATTTGTCTGG	509	*lmo1030*	Ryu et al. (2013)
	lmo1030-R	ACCATCCGCATATCTCAGCCAACT			

5. Multiplex PCR mixture

Single PCR was performed to evaluate the primer specificity with the variable conditions, which ensured that all primer pairs would work under the same PCR conditions. Upon finalization, the multiplex PCR assay was optimized to enable the detection of PCR amplicons produced by 4 primer pairs simultaneously. The multiplex PCR assay consisted of a 25 µl total volume with the mixture of 12.5 µl of Takara Premix *Ex* Taq™, 1 µl of both eaeAF/eaeAR primers (10 pmol), 1 µl of each of the 3 remaining primer pairs (30 pmol for 16SFa/16SR, 5 pmol for STM3098-f2/STM3098-r2, and 5 pmol for lmo1030-f/lmo1030-r), 3 µl of the three, evenly mixed DNA templates, and then water to volume.

Table 3. Multiplex PCR mixture composition

Component	Volume	Concentration
gDNA*	3 µl	10 ng
16SFa	1 µl**	30 pmol
16SR	1 µl**	30 pmol
eaeAF	1 µl**	10 pmol
eaeAR	1 µl**	10 pmol
STM3098-f2	.5 µl**	5 pmol
STM3086-r2	.5 µl**	5 pmol
lmo1030-f	1 µl**	5 pmol
lmo1030-r	1 µl**	5 pmol
Ex-*Taq* Polymerase	12.5 µl	
DNase & RNase free H_2O	2.5 µl	
Total	**25 µl**	

***** gDNA represents the 3 µl that was the evenly mixed with gDNA from *L. monocytogenes* ScottA, *S.* Typhimurium LT2, *E. coli* O26:H11.
****** For multiplex PCR, each assay contained all four primer pairs.

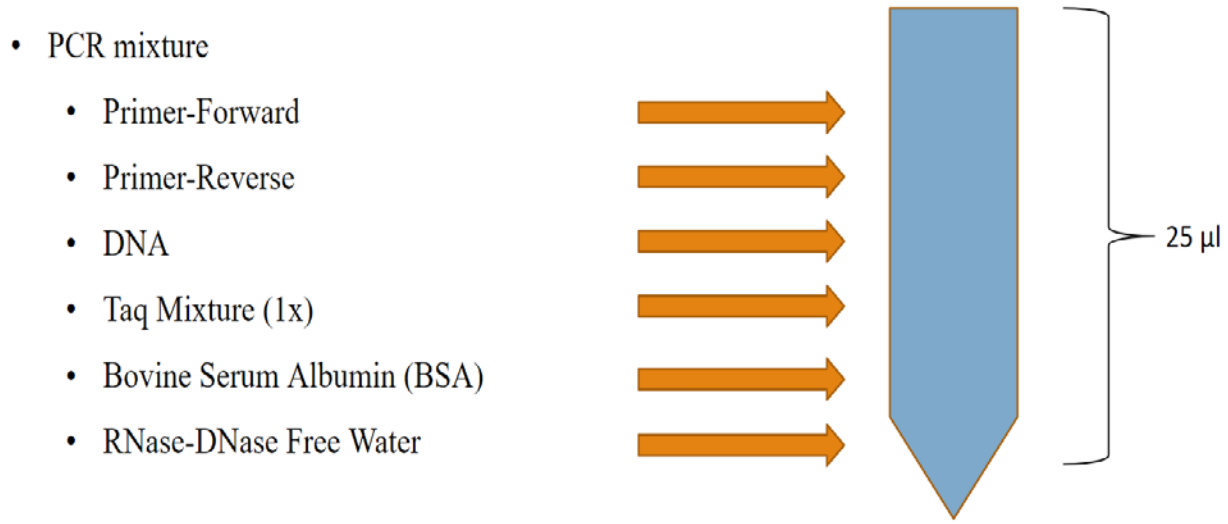


Figure 1. Graphical representation of the standard procedure of the multiplex PCR assay utilized in this research.

6. PCR conditions

The PCR assay has been optimized to conditions of 94 °C for 5 min (pre-denaturation), and then 35 cycles of 94 °C for 30 s (denaturation), 65 °C for 30 s (annealing), and 72 °C for 45 s (extension), with a final extension cycle at 72 °C for 5 min, and a final hold of 4 °C. Single PCR was then carried out alongside multiplex PCR, which allowed for a set of positive controls to confirm that all PCR amplicons were displayed properly. Different agarose gel (SeaKem LE Agarose, Lonza, Rockland, ME, USA) concentrations (1, 1.5, and 3%) were tested for electrophoresis to confirm band discrimination clearly. A 3% of agarose gel was selected as the primary percentage to be used in all future trials, in conjunction with electrophoresis run at 100 V for 40 min. All agarose gels were stained with Gel Red Dye (10 mg/ml) and transilluminated using a Molecular Imager™ Gel Doc XR+© Imaging System (Cambridge Scientific Products, Waterland, MA, USA).

7. Produce trials

In order to evaluate the detection limit of the optimized multiplex PCR assay, 3 pathogens were spiked to kale and romaine lettuce, respectively. *Listeria monocytogenes* ScottA, *S*. Typhimurium LT2, and *E. coli* O26:H11 were grown respective media, followed by colony isolation with subsequent inoculation into 5 ml of TSB in 15 ml tubes. Broths were incubated at 37 °C for 24 h to achieve approximately 10^9 CFU/ml. A 1 ml of each grown culture were diluted serially to 10^{-8} in phosphate-buffered saline (PBS). A 25 g of each kale or romaine lettuce was tested for initial bacterial loads using the FDA's Bacterial Analysis Manual (BAM), 3M™ aerobic plate count (APC), and coliform petrifilms. Simultaneously, 25 g of each produce (kale and romaine lettuce) was weighed individually, placed into stomacher bags, and spiked 1 ml aliquots of serially diluted pathogens (10^0 to 10^{-8}). This made for a total of 74 bags (triplicates of 9 for each single pathogen, 9 of all 3 pathogens, for both romaine and kale + a negative control bag for each). Bags were allowed to stand before addition of 225 ml of buffered peptone water, followed by placed into a Stomacher® 400 Circulator (Seward Ltd, Worthing, West Sussex, UK) for 1 min. All bags were separated based on produce and pathogen and placed into respective incubators at 37 °C. A 1 ml of aliquots were collected in triplicates from each bag at timepoints 0, 2, 4, 6, 8 and 18 h incubation. These aliquots were placed into marked centrifuge tubes and immediately frozen for later use.

8. DNA extraction from produce

For DNA extraction from the fresh produce samples, they were thawed, pelleted by centrifugation in a Centrifuge 5424 R (Eppendorf) at 16,000 rpm for 3 min, and 900 µl of supernatant was discarded. The pellet was resuspended with 100 µl of supernatant. Genomic DNA was then isolated via a boiling method. A 150 mg of 0.1 mm Precellys 24 Glass Lysing Beads (Bertin Technologies, Paris, France) was added to each tube followed by vortexing with a Vortex Genie® 2 (Scientific Industries Inc) using a bead beater attachment for 30 sec. Samples were then placed into a boiling water bath using an Elite Cuisine Hotpot Model EHP-001 (Maximatic, Industry, CA, USA) for 10 min, followed by placed to crushed ice for 10 min. They

were then removed and placed into a centrifuge at 16,000 rpm for 2 min. A 100 µl of supernatant including DNA was transferred to a fresh tube for subsequent use as a template DNA.

9. Single and multiplex PCR assays with produce samples

The single and multiplex PCR assays were applied to assess the detection limit of 3 pathogens recovered from fresh produce samples using the optimized and established conditions defined above. The extracted template DNA was used in place of the original pure culture gDNA. A 1.6 µl (20 mg/ml) of Bovine Serum Albumin (BSA) was added to the PCR mixture to increase sensitivity, and water volume was reduced to maintain adequate ratios. 3% agarose gel, stained with Gel Red Dye, was used for gel electrophoresis, at 100 V, for 40 min and viewed using a UV transilluminator.

SIGNIFICANT ACCOMPLISHMENTS:

1. Optimization of PCR conditions

- The PCR conditions have been continuously developed since the onset on the project, with the goal of achieving optimized conditions. Both a distinctive clarity of PCR amplicons produced by the PCR assay and an overall preventive of unspecific bands/primer dimer pairings during PCR assays are important.
- See optimized PCR mixture and conditions addressed above.

2. Specific primer pairs for the detection of *L. monocytogenes* ScottA, *S.* Typhimurium LT2, and *E. coli* O26:H11 have been selected.

- A variety of primer pairs have been evaluated and undergone various conditions in order to amplify only target bacterial species and ensure that they do not compete with other species.
- The selected primer pairs also exhibited a minimum of 72 bp difference in the PCR product size, which allow for proper separation of bands during agarose gel electrophoresis, and ease of evaluating results.
- The current primer pairs have been proven to work appropriately with one another, under all current PCR conditions and parameters set forth by the current state of the research.

3. PCR result and subsequent electrophoresis have become consistent

- Techniques utilized for the genomic DNA extraction, PCR mixture composition and condition, and agarose gel electrophoresis were become consistent throughout research progress.

- All PCR procedure exhibited the reproducibility, stability, and lack of deviation, when collecting data upon the completion of research.

4. Optimization of multiplex PCR assay

- EAE-a/b primer pair produced 384 bp of amplicon were replaced with eaeAF/AR primer pairs to overcome distinct band distance and competition with other primer pairs.
- This new primer pair allows for a constant band intensity between all positive bands when tested in both single and multiplex PCR assays (Figure 2).

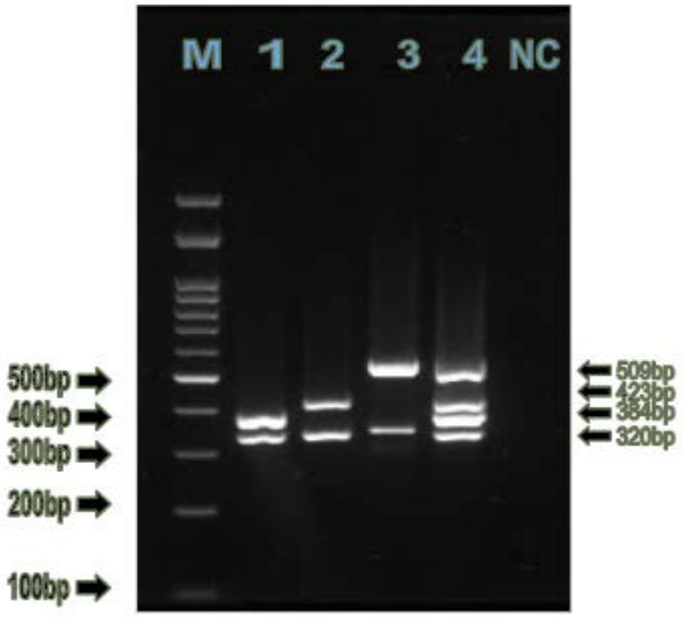


Figure 2. The optimized multiplex PCR assay with *L*. *monocytogenes* ScottA, *S*. Typhimurium LT2, and *E*. *coli* O26:H11. The 3% agarose gel was run at 100 V for 45 min. Lane M: 100 bp DNA ladder, Lane 1: *E*. *coli* O26:H11, Lane 2: *S*. Typhimurium LT2, Lane 3: *L*. *monocytogenes* ScottA, Lane 4: *E*. *coli* O26:H11, *S*. Typhimurium LT2, and *L*. *monocytogenes* ScottA, Lane NC: negative control.

5. Sample inoculation and acquisition was completed with no detectable contamination

- 74 bags containing either kale or romaine lettuce with their respective pathogens, were inoculated, incubated and sampled over an 18 h period.
- Throughout subsequent testing with kale and romaine lettuce, there was no natural contamination of 3 pathogens used in this study.

6. Single PCR assay with spiked fresh produce samples have been completed

- Single PCR assay for all samples has been completed.

- Kale: Detection limit is from 10^0 to 10^{-3} at 0 h and increased steadily throughout the time points. *E. coli* O26:H11 can be detected in all samples at 18 h of incubation time (Figure 3).
- Kale: Detection limit is from 10^0 to 10^{-4} at 0 h and increased steadily throughout the time points. *S.* Typhimurium LT2 can be detected in all samples at 18 h of incubation time (Figure 4).
- Kale: Detection limit is from 10^0 to 10^{-1} at 0 h and increased steadily throughout the time points. *L. monocytogenes* ScottA can be detected 10^{-3} at 18 h of incubation time (Figure 5).
- Romaine Lettuce: Detection limit is from 10^0 to 10^{-2} at 0 h and increased steadily throughout the time points. *E. coli* O26:H11 can be detected in all samples at 18 h of incubation time (Figure 6)
- Romaine lettuce: Detection limit is from 10^0 to 10^{-3} at 0 h and increased steadily throughout the time points. *S.* Typhimurium LT2 can be detected in all samples at 18 h of incubation time (Figure 7).
- Romaine lettuce: Detection limit is from 10^0 to 10^{-1} at 0 h and increased steadily throughout the time points. *L. monocytogenes* ScottA can be detected 10^{-3} at 18 h of incubation time (Figure 8)

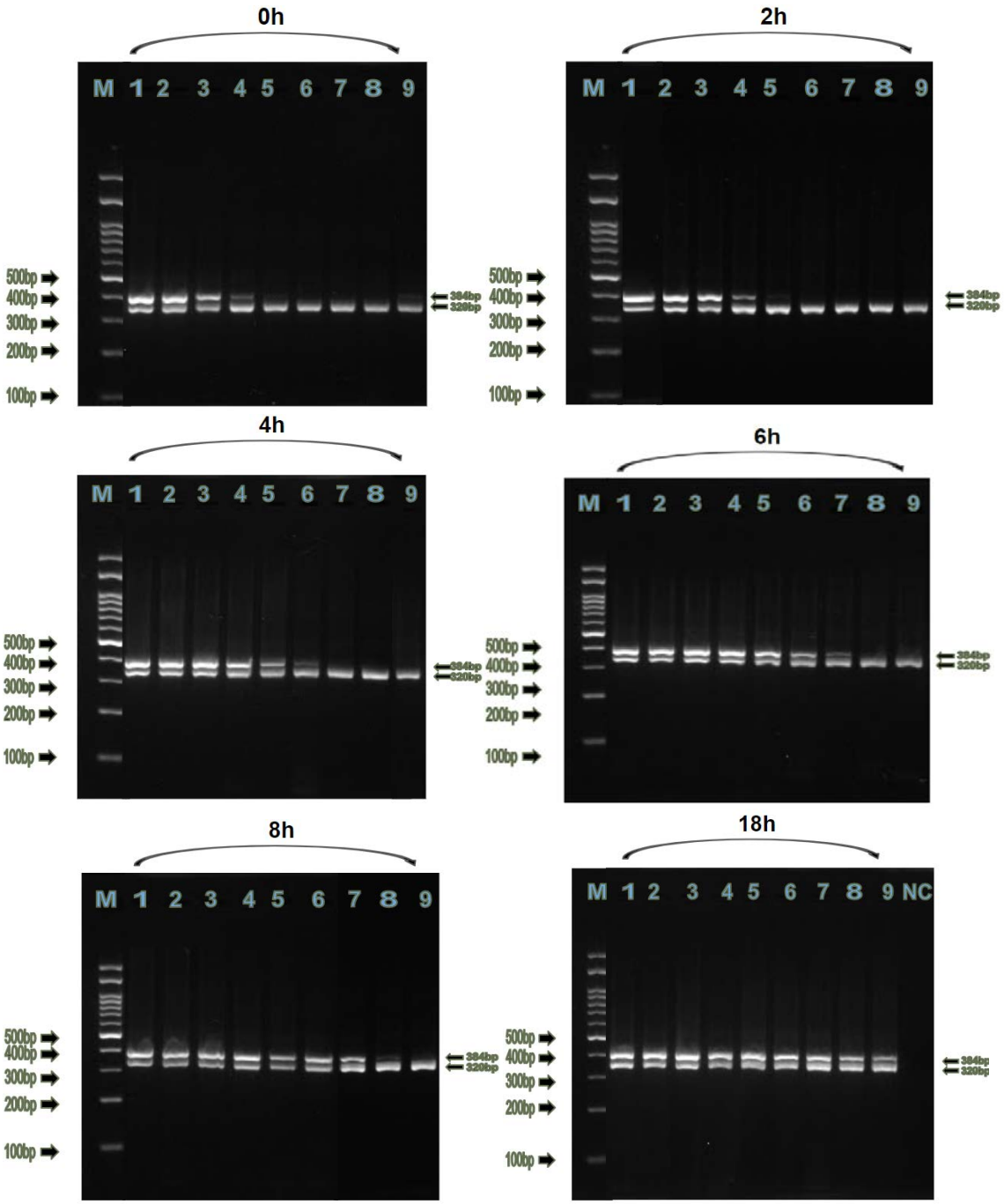


Figure 3. Single PCR with kale samples inoculated with *E. coli* O26:H11 from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *E. coli* O26:H11 from 10^{0} to 10^{-8}, Lane NC: Negative control, 320 bp band represented 16S (internal control), 384 bp band represented *E. coli* O26:H11.

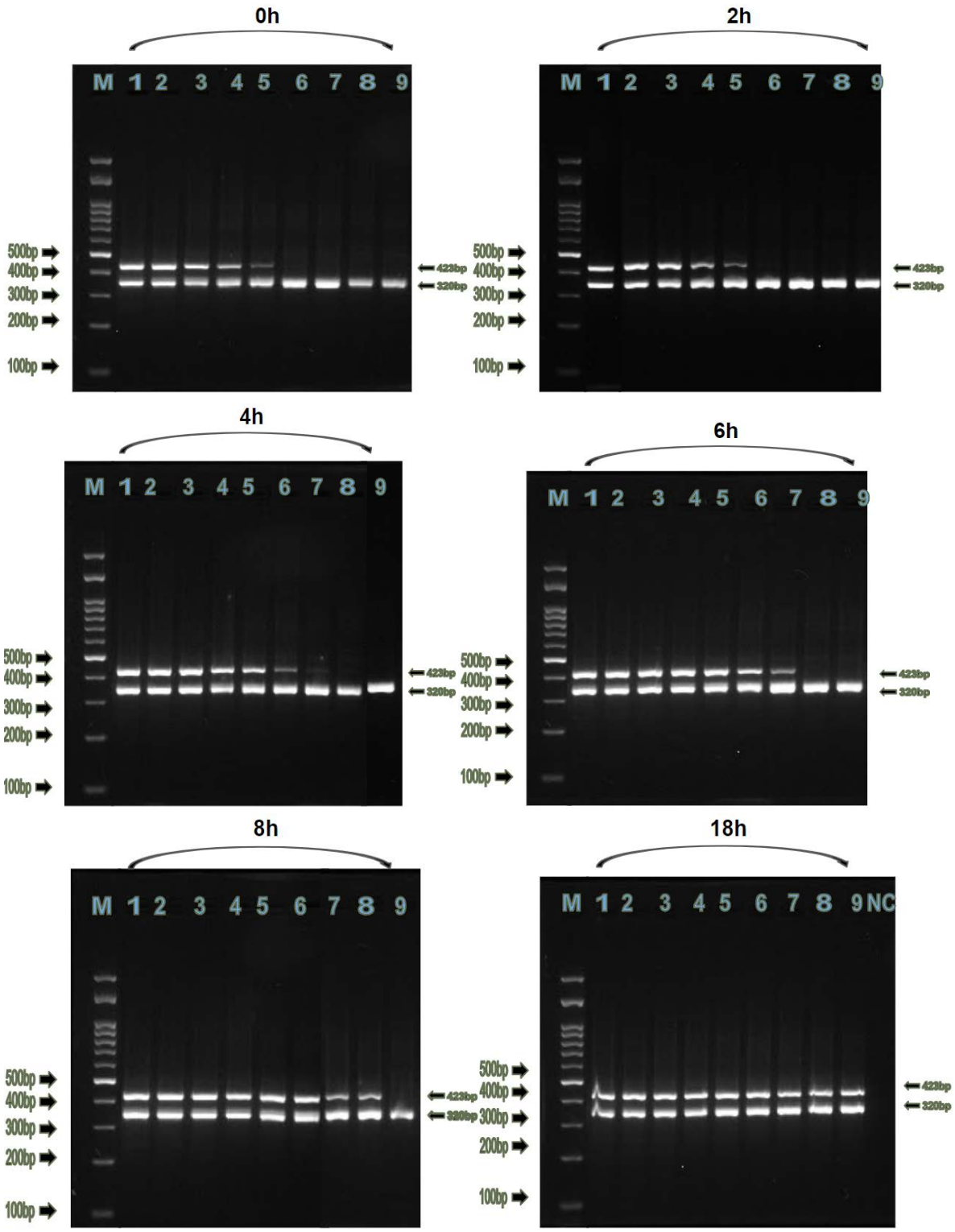


Figure 4. Single PCR with kale samples inoculated with *S*. Typhimurium LT2 from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *S*. Typhimurium LT2 from 10^{0} to 10^{-8}, Lane NC: Negative control, 320 bp band represented 16S (internal control), 423 bp band represented *S*. Typhimurium LT2.

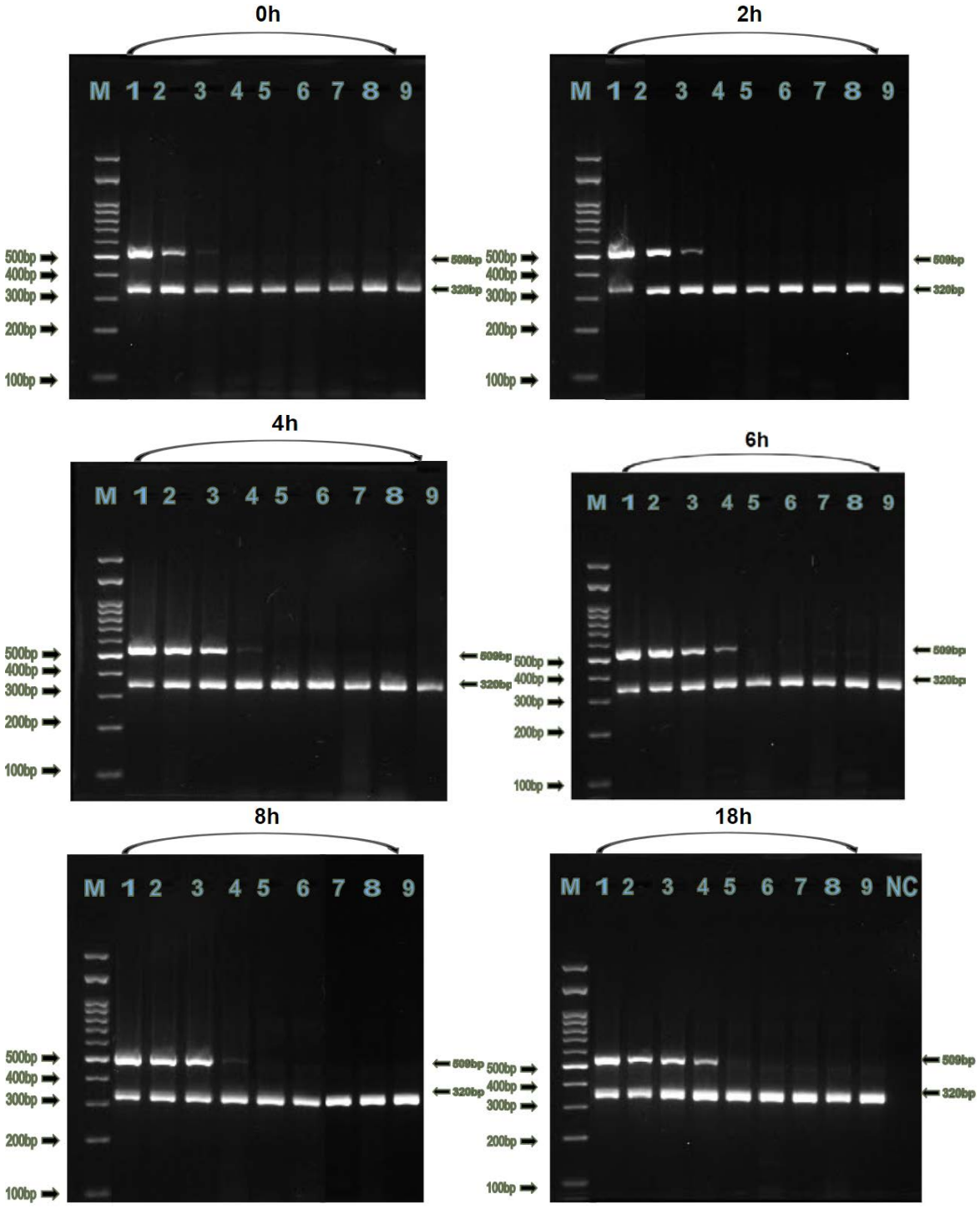


Figure 5. Single PCR with kale samples inoculated with *L. monocytogenes* ScottA from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *L. monocytogenes* ScottA from 10^{0} to 10^{-8}, Lane NC: Negative control, 320 bp band represented 16S (internal control), 509 bp band represented *L. monocytogenes* ScottA.

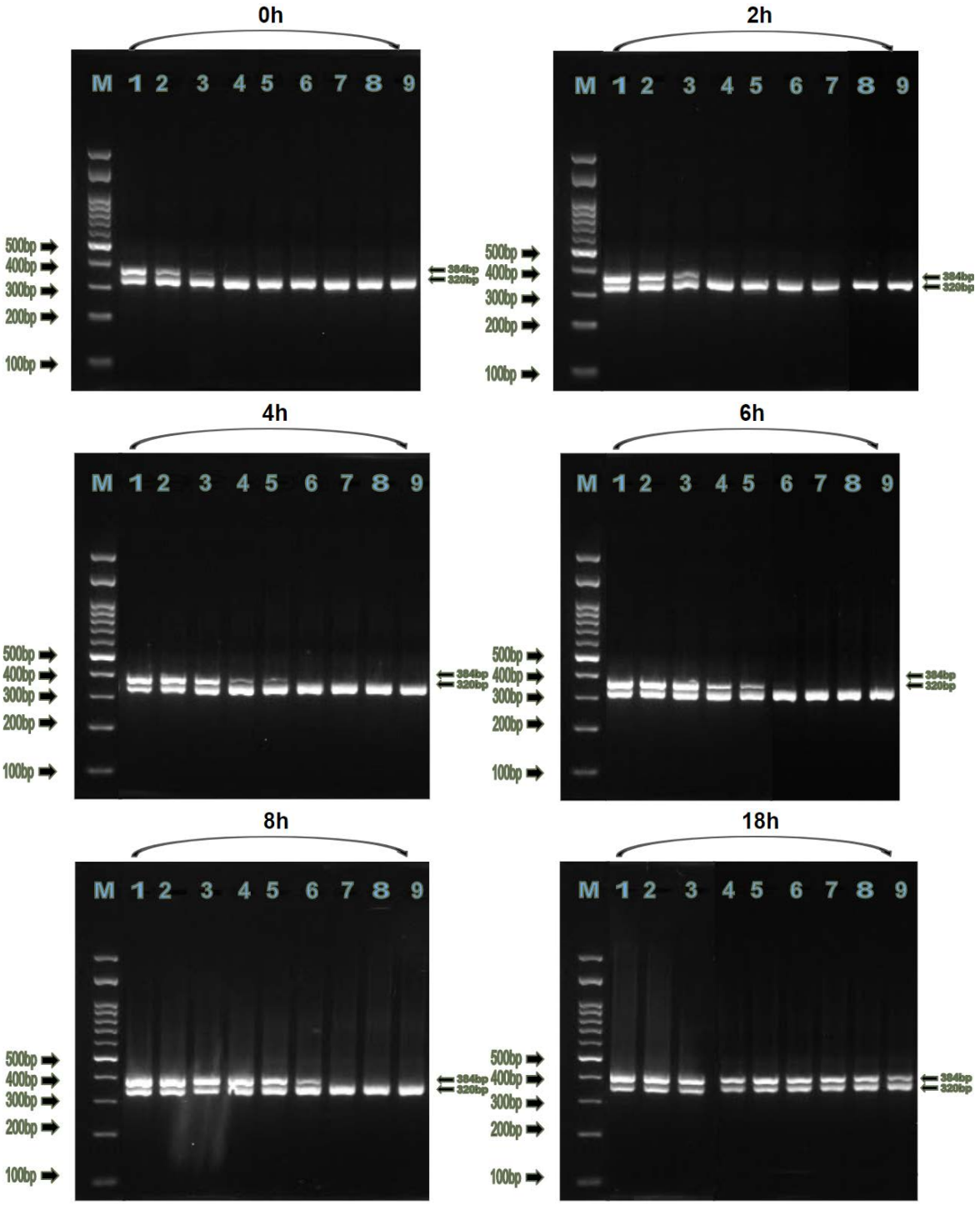


Figure 6. Single PCR with romaine lettuce samples inoculated with *E. coli* O26:H11 from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *E. coli* O26:H11 from 10^0 to 10^{-8}, Lane NC: Negative control, 320 bp band represented 16S (internal control), 384 bp band represented *E. coli* O26:H11.

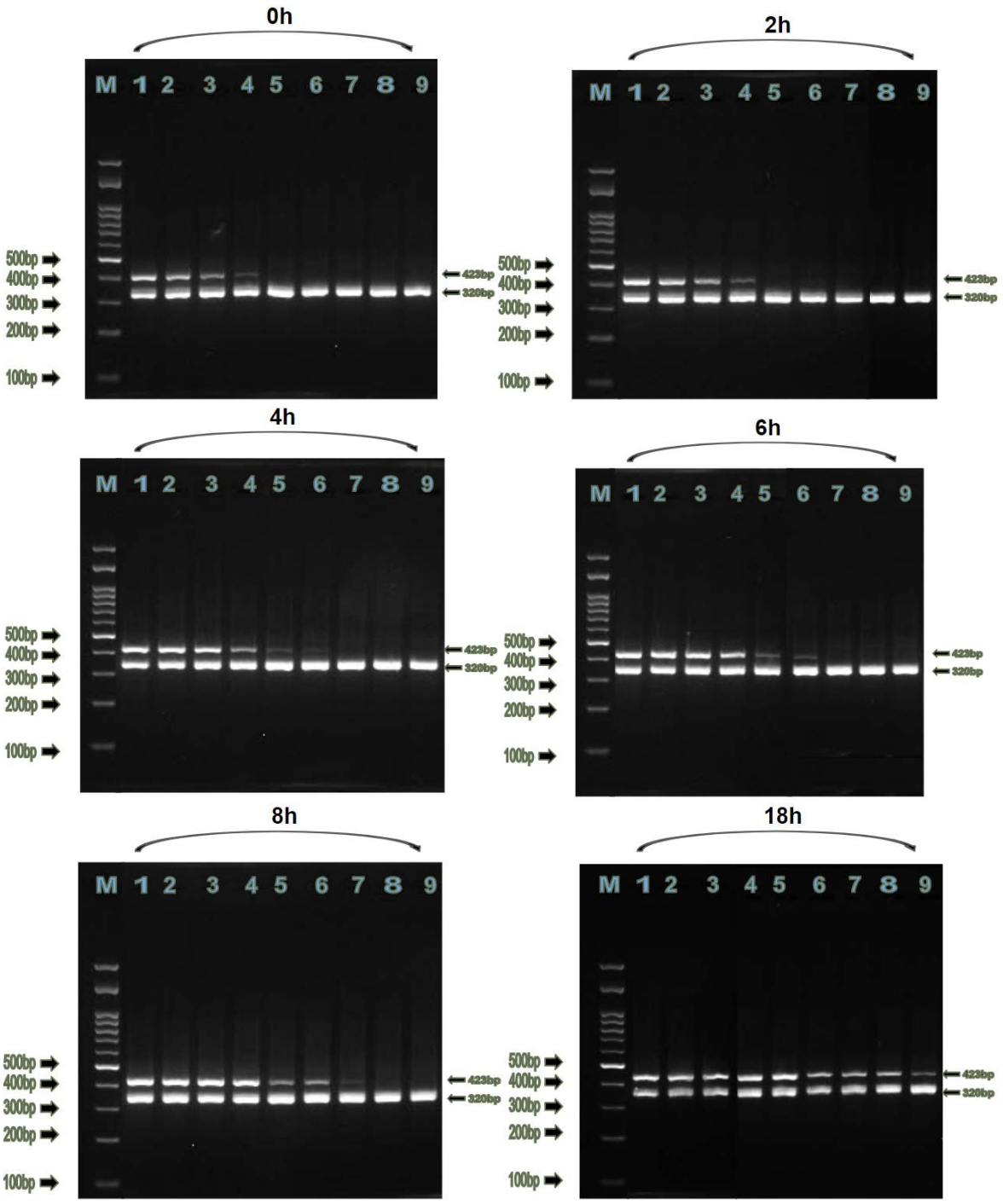


Figure 7. Single PCR with romaine lettuce samples inoculated with *S*. Typhimurium LT2 from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *S*. Typhimurium LT2 from 10^{0} to 10^{-8}, Lane NC: negative control, 320 bp band represented 16S (internal control), 423 bp band represented *S*. Typhimurium.

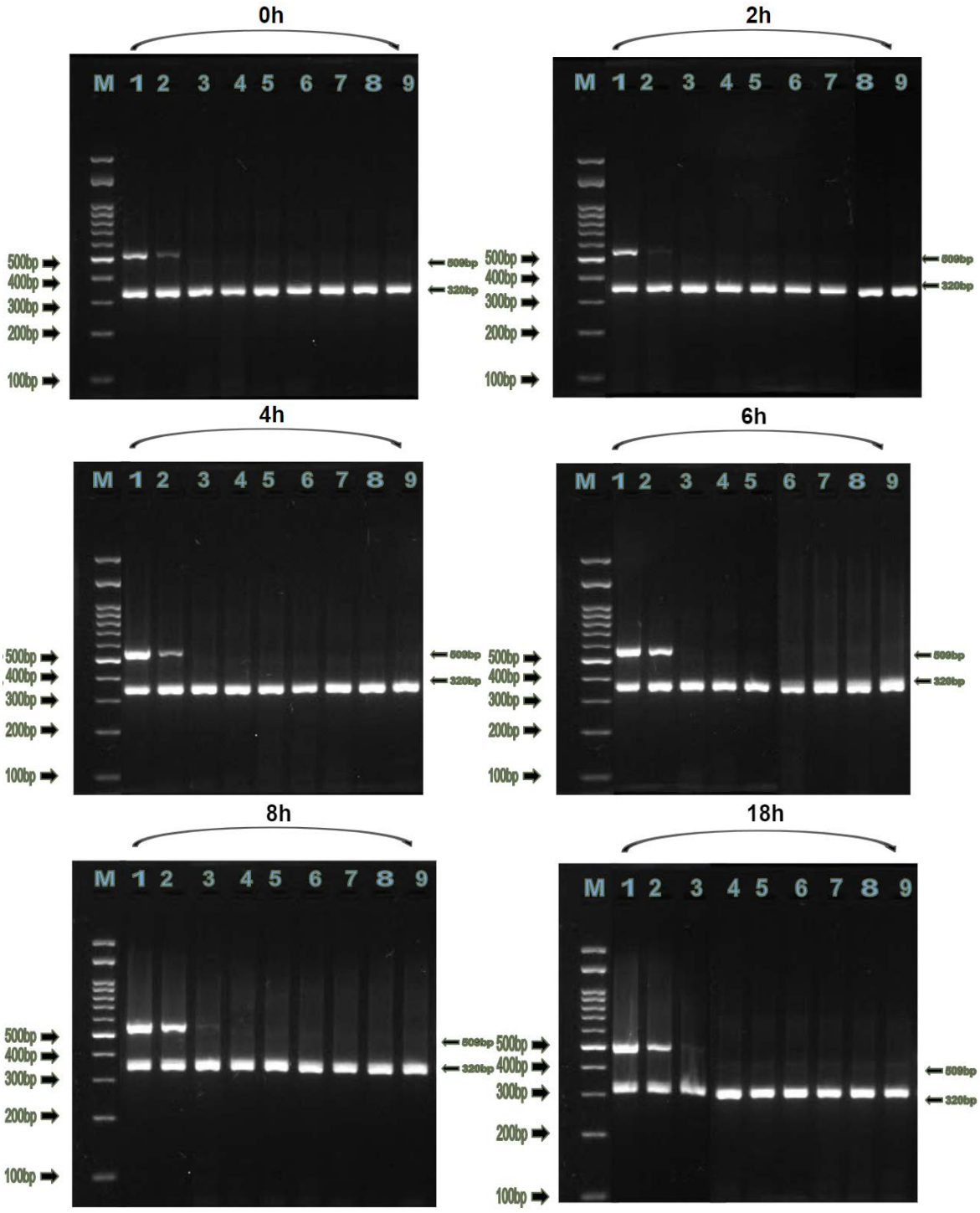


Figure 8. Single PCR with romaine lettuce samples inoculated with *L. monocytogenes* ScottA from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder; Lanes 1 to 9: Serially diluted *L. monocytogenes* from 10^{0} to 10^{-8}, Lane NC: Negative control, 320 bp band represented 16S (internal control), 509 bp band represented *L. monocytogenes* ScottA.

7. Multiplex PCR assay with spiked fresh produce samples has been completed.

- The optimized multiplex PCR assay can simultaneously detect 3 foodborne pathogens (*L*. *monocytogenes* ScottA, *S*. Typhimurium LT2, and *E*. *coli* O26:H11) in fresh produce (kale and romaine lettuce).
- Gram-positive *L*. *monocytogenes* ScottA showed a low detection limit in fresh produce samples.
- Kale: Detection limit is from 10^0 to 10^{-3} for *E*. *coli* O26:H11, 10^0 to 10^{-4} for *S*. Typhimurium LT2, and 10^0 for *L*. *monocytogenes* ScottA at 0 h, respectively, and increased steadily throughout the time points. *S*. Typhimurium LT2 and *E. coli* O26:H11 can be detected in all samples at 18 h of incubation time except *L*. *monocytogenes* ScottA (10^{-1}) (Figure 9).
- Romaine lettuce: Detection limit is from 10^0 to 10^{-2} for *E*. *coli* O26:H11, 10^0 to 10^{-3} for *S*. Typhimurium LT2, and 10^0 for *L*. *monocytogenes* ScottA at 0 h, respectively and increased steadily throughout the time points. *S*. Typhimurium LT2 and *E. coli* O26:H11 can be detected in all samples at 18 h of incubation time except *L*. *monocytogenes* ScottA (10^0) (Figure 10).

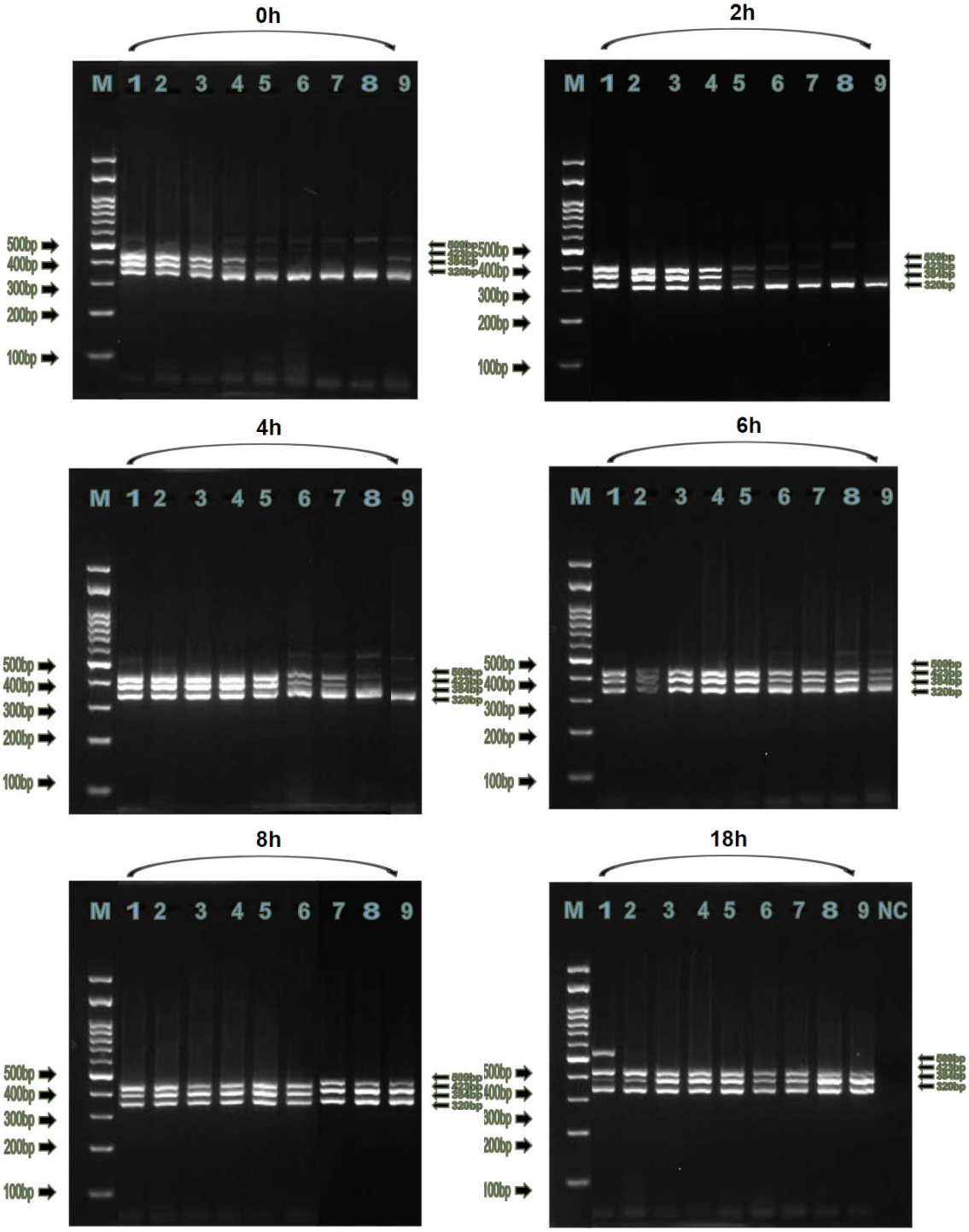


Figure 9. Multiplex PCR assay for kale inoculated with all three pathogens (*L*. *monocytogenes* ScottA, *S*. Typhimurium LT2, and *E. coli* O26:H11) from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder, Lanes 1 to 9: Serially diluted *L*. *monocytogenes* ScottA, *S*. Typhimurium LT2, and *E. coli* O26:H11 from 10^{0} to 10^{-8}. 320 bp band represented 16S (internal control), 384 bp band represented *E*. *coli* O26:H11, 423 bp band represented *S*. Typhimurium LT2, and 509 bp band represented *L. monocytogenes* ScottA, Lane NC: Negative control.

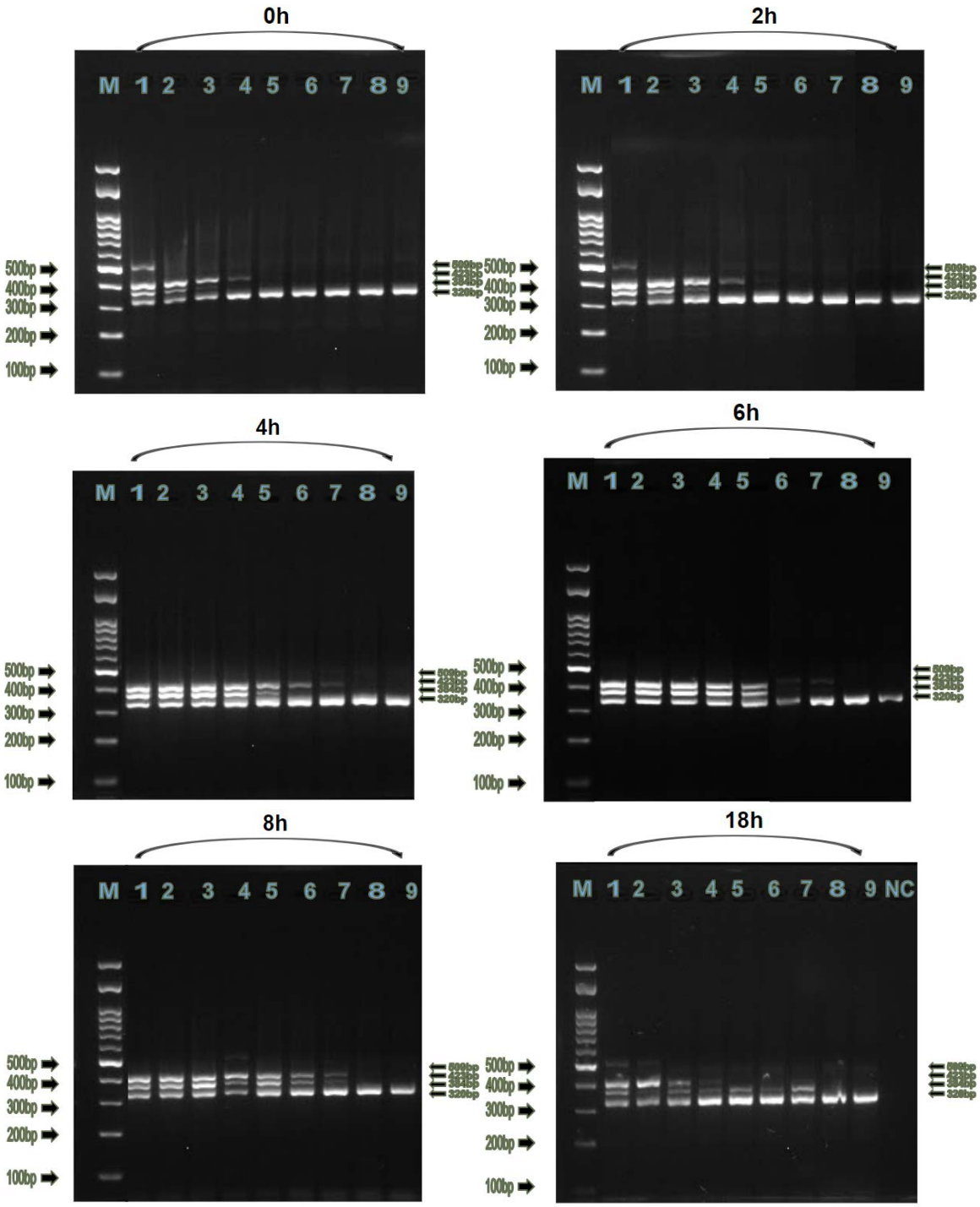


Figure 10. Multiplex PCR assay for romaine lettuce inoculated with all three pathogens (*L. monocytogenes* ScottA, *S*. Typhimurium LT2, and *E. coli* O26:H11) from 0 to 18 h of incubation. Lane M: 100 bp DNA ladder, Lanes 1 to 9: Serially diluted *L. monocytogenes* ScottA, *S*. Typhimurium LT2, and *E. coli* O26:H11 from 10^{0} to 10^{-8}. 320 bp band represented 16S (internal control), 384 bp band represented *E. coli* O26:H11, 423 bp band represented *S*. Typhimurium LT2, and 509 bp band represented *L. monocytogenes* ScottA, Lane NC: Negative control.

BENEFITS & IMPACT:

The Centers for Disease Control and Prevention (CDC) have estimated that 48 million cases of foodborne illnesses occur in the United States (US) annually and approximately 128,000 cases require hospitalization and 3,000 cases result in death. The CDC reported that viruses are major causative agents for foodborne illnesses (59%), followed by bacteria (39%), and parasites (2%); however, bacterial agents are associated with the more severe cases, being responsible for most of the hospitalizations (63.9%) and deaths (63.7%).

Polymerase chain reaction (PCR) is one of the molecular techniques to amplify a specific portion of nucleic acids (DNA or RNA) in organisms for identification has been widely utilized to detect targeted bacteria specifically in foods. In general, one copy of DNA can be increased to 100 million copies within 3 hours via PCR. Since multiplex PCR can amplify specific DNA sequences and discriminates each target strain in a sample simultaneously, considerable time and cost can be saved (reduce the total assay time to within 3 h, multiple assays at one time and 60% less cost versus a single PCR-based assay). The designed multiplex PCR assay has displayed its ability to detect all the pathogens when on an individual basis, and *E. coli* and *Salmonella* when they are together. It falls short on detecting all three simultaneously, but a case of having all three pathogens together in a commercial environment is unlikely, given the growth conditions and stipulations for each.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Based on the preliminary data generated from the ARF grant, PI (Dr. Si Hong Park) has received 3 funds below.

	Title	**Agency**	**Amount**	**Period**	**Role**
1	Metagenomic analysis of stress resistant *Listeria monocytogenes* isolated from foods	Korea Food Research Institute (KFRI)	*$120,751	04/01/19 to 12/31/21	PD
2	Investigation of microbiome shift by plant probiotic in strawberry plant	Oregon Department of Agriculture (ODA)	$174,527	10/01/19 to 09/30/21	PD
3	Application of molecular-based assays for foodborne pathogens detection and indicator bacteria identification associated with food safety and quality	Korean-American Scientists and Engineers Association (KSEA)	$10,000	08/01/18 to 7/31/19	PD

* Total fund amount might be changed based on the currency exchange rate.

FUTURE FUNDING POSSIBILITIES:

Dr. Si Hong Park will apply the United States Department of Agriculture - Agriculture and Food Research Initiative (USDA-AFRI) grant

REFERENCE:

Harris, K.A. and Hartley, J.C. 2003. Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. Journal of medical microbiology, 52: 8, 685-691.

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