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Research Article

The *pipB* Gene as Target for Development of Detection Method of Pathogenic Bacteria *Salmonella typhi* Using Real-time Polymerase Chain Reaction

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ABSTRACT

Salmonella typhi is a bacteria that leads to typhoid fever and one of the causes of death due to bacteria infections. In Indonesia, typhoid fever occurs around 1,100 cases per 100,000 population per year, with a mortality rate of 3.1-10.4%. It's necessary to develop a rapid and accurate detection of *Salmonella typhi*. The *pipB* gene of *Salmonella typhi* has the function of being an autophagia inhibitor in humans. This study aims to develop a detection kit for *Salmonella typhi* pathogenic bacteria targeting the *pipB* gene using a *pipB* primer in confirmation, specificity, and sensitivity tests. The results showed that *pipB* primer can amplify *Salmonella typhi* DNA fragment of 196 bp at the optimum annealing temperatures between 54-62°C. Confirmation test with real-time PCR found that the *pipB* primer pair (*pipB*-F and *pipB*-R) amplified at cycle 12.93 and 13.10 (Duplo) with a Tm value of 84.05°C and 84.20°C (Duplo). Based on the difference and average value produced in the confirmation and specificity test, *pipB* primer has amplified *Salmonella typhi* DNA at Ct 12.47±0.6 with a Tm value of 83.62°C±0.6. The *pipB* primer pair (*pipB*-F and *pipB*-R) could distinguish target from non-target bacteria based on their cycle threshold (Ct) and melting temperature (Tm) values. The primer design of *pipB* primer pair (*pipB*-F and *pipB*-R) successfully detected *Salmonella typhi* bacteria with the smallest concentration of 55.78×10^2 CFU equivalent to 3.2 pg/µL. Based on the results, *Salmonella typhi pipB* primer successfully detected *Salmonella typhi* bacteria DNA rapidly, specifically, and sensitively using the real-time polymerase chain reaction method.

1. Introduction

Food is one of the basic needs of human life. Unsafe food can cause a disease called foodborne disease. Foodborne disease is a disease that arises from consuming food containing toxic materials or compounds (intoxication) or due to contamination of pathogenic microorganisms (infection) (Srinivasan *et al.* 2020). One of the bacteria associated with food poisoning is *Salmonella* spp. *Salmonella* infects more

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than 1 million people every year, and 380 people die of it (Scallan *et al.* 2011). *Salmonella typhi* is a bacteria that causes typhoid disease in humans. Typhoid disease is a disease that originates from an intestinal infection caused by the entry of *Salmonella typhi* bacteria into the body through food or drink contaminated with a certain amount of these bacteria. The epidemiology of typhoid disease shows that the disease has spread almost all over the world and is an endemic disease often found in developing countries (Nurjayadi *et al.* 2011).

A molecular biology technique used to amplify specific DNA sequences into thousands to millions of copies of DNA sequences is called Polymerase Chain Reaction (PCR) (Hewajuli & Dharmayanti 2014). PCR has 30-40 cycles, and three main stages are denaturation, annealing, and extension (Pestana *et al.* 2010). PCR is one of the molecular methods that is sensitive enough to detect infectious diseases, one of which is foodborne diseases (Widyastuti 2017). Compared to conventional PCR, real-time PCR has higher specificity and sensitivity. The real-time PCR method requires fluorescence dye to produce real-time fluorescence data for quantification analysis. Real-time PCR techniques are more efficient and effective than conventional PCR (Kasajima *et al.* 2004).

In previous studies, a fast, accurate, effective, sensitive, and specific detection using real-time PCR method on *Salmonella typhi* bacteria targeting the *fimC* gene in contaminated egg food samples was developed. Further research on other genes besides *fimC* in *Salmonella typhi* may provide deeper insights into the pathogenesis mechanism of *Salmonella typhi* bacteria. Research on genes involved in cell adhesion or invasion may help explain how *Salmonella typhi* infects the host and survives in the human immune system (Nurjayadi *et al.* 2019). The *pipB* gene plays a role in *Salmonella typhi* infection through its ability to facilitate adhesion, evade immune responses, and contribute to bacteria virulence (Zhang *et al.* 2008). Continuing research on other pathogenic genes in *Salmonella typhi* is essential to track epidemic spread and infection patterns to design more effective public health interventions in countries with a high incidence of typhoid, such as Indonesia.

Therefore, this study was conducted to develop a detection method for *Salmonella typhi* that targets the pipB gene using the real-time Polymerase Chain Reaction method. The *pipB* gene is located in the Salmonella Pathogenicity Island (SPI) region, which also plays a role in causing human disease. *Salmonella typhi* has a genome length about 5 million base pairs (bp) and encodes about 4,000 genes. Of these 4,000 genes, more than 200 genes are functionally inactive. Genes for virulence factors are found on the Pathogenicity Island (PI). Two major PIs encode type three secret systems (TTSS) that translocate bacteria virulence proteins into host cells during infection (Zhang *et al.* 2008). The stage of this research can proceed to bacteria detection using the real-time Polymerase Chain Reaction method in food samples.

2. Materials and Methods

2.1. Primer of the *pipB* Design

Primer was designed using the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/) and the Primer-Basic Local Alignment Search Tool (Primer-BLAST) program to identify specific primers in *Salmonella typhi* bacteria. Primer pairs selected according to the criteria as good primer pairs will be re-analyzed using NetPrimer program (https://www.premierbiosoft.com/netprimer/) and OligoAnalyzer program (https://www.idtdna.com/oligoanalyzer) to determine the secondary structure. The *pipB* gene-designed primer pair is in 876 bp size and in the order of 1,086,906-1,087,781 bp. The sequences of primer pairs for the forward and reverse *pipB* gene are shown below (Table 1), with an amplicon length target of 196 bp. The primer was designed in the Macrogen Synthesis, Inc.-Korea commercial laboratory.

2.2. Inoculation of *Salmonella typhi* Bacteria

Bacteria samples of *Salmonella typhi* from Glycerol Stock were cultured on a Salmonella-Shigella Agar (SSA) (Microbiology Lab, UI) plate using the spread method, which was later incubated for 24 hours 37°C (overnight culture). Single black colonies formed on agar were inoculated in Luria-Bertani (LB) broth (Marck), taken using a sterile inoculation loop, and incubated (Orbital Shaking Incubator LM-400D) under an aeration shaker at 150 rpm at 37°C for 18 hours.

Table 1. Primer pairs sequence of *Salmonella typhi pipB* gene

Bacteria	Gene	Forward	Reverse
Salmonella typhi	*pipB*	5'-GGGAGTGGA GTAGGGGTATG-3'	5'-AAGCAAACCTG ACTCACGCA-3'

2.3. *Salmonella typhi* DNA Extraction

1.5 ml of *Salmonella typhi* culture from Luria-Bertani broth was transferred into 2 ml micro tubes and centrifuged (Sorvall™ Legend™ Micro 17R Microcentrifuge) at 5,000×g for 5 minutes. After that, the pellet containing *Salmonella typhi* bacteria DNA was isolated using Geno Plus Genomic DNA Extraction Miniprep System isolation kit (Viogene) with DNA isolation preparation for gram-negative bacteria to obtain pure isolates containing *Salmonella typhi* bacteria DNA. In the isolation process, two elutions were carried out to ensure that DNA had been extracted from the column, thus maximizing the overall extraction results. Then, A quantitative test of DNA isolate was retrieved by measuring the purity and concentration of bacteria DNA isolate using NanoDrop Spectrophotometer (NanoVue™ Plus Spectrophotometer) as much as 2 µL of DNA isolate at wavelength A260/A280. *Salmonella typhi* bacteria DNA isolates were then qualitatively tested with 0.7% agarose gel electrophoresis using Green Safe. Electrophoresis results were analyzed using a UV Transilluminator instrument (Vilber Lourmat) to visualize the isolated DNA. *Salmonella typhi* bacteria DNA isolates were stored at -20°C.

2.4. Optimization of the Annealing Temperature of the *pipB* Primer

Optimization of the annealing temperature was carried out to determine the optimal temperature in the annealing process. The optimization stage was carried out by gradient PCR instrument (TaKaRa PCR Thermal Cycler) over the temperature range of 54-62°C to amplify the pipB gene of *Salmonella typhi* bacteria DNA. This test used 25 µL of reaction mix containing purified *Salmonella typhi* typhi DNA isolate, *pipB* forward and reverse primers, Nuclease Free Water (NFW), and NZYTaq II 2x Green Master Mix. Furthermore, the amplification stage was carried out with initial denaturation, denaturation, annealing, and extension stages. The temperature and time required during the PCR process are 95°C for 100 seconds, 95°C for 30 seconds, 54-62°C for 30 seconds, and 72°C for 60 seconds, respectively. The Amplification process was repeated for 35 cycles. The electrophoresis process using 2% agarose gel was then used to analyze the results of DNA amplification. The electrophoresis process requires TAE 1x buffer as a solvent and florovue in the form of GreenSafe as a colorant. Electrophoresis requires an electric current of 400 A with a voltage of 70 V for 4,200 seconds.

2.5. Confirmation Test of *Salmonella typhi*

The confirmation test was conducted to determine the ability of primer to amplify target bacteria DNA by real-time PCR method. The confirmation test with the real-time PCR method used 40 cycles to produce the Ct (cycle threshold) value on the amplification curve and the melting temperature (Tm) value on the melting curve. The *pipB* primer confirmation test to detect *Salmonella typhi* bacteria using the real-time PCR (Mic qPCR Cycler Bio Molecular Systems) method used 20 µL of reaction mixture consisting of ExcelTaq 2X qPCR Master Mix, forward and reverse *pipB* primers, pure isolate of *Salmonella typhi* DNA, and Nuclease Free Water (NFW). The *pipB* primer confirmation test on *Salmonella typhi* was performed in Duplo to identify errors that may occur during the analysis process. The real-time PCR process includes 4 important steps that are repeated in 40 cycles, namely initial denaturation, denaturation, annealing, and extension. The temperature and time required during the real-time PCR process are 95°C for 180 seconds, 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 420 seconds, respectively.

2.6. Specificity and Sensitivity Test

The specificity test was conducted to ensure that the *pipB* gene primer that has been specifically designed can only detect and amplify *Salmonella typhi* bacteria. The primer specificity test used *Salmonella typhi* as a positive control and several non-target bacteria to determine the specificity of the primer. The sensitivity test aims to produce a standard curve to determine the Limit of Detection (LoD) of pipB primer in detecting *Salmonella typhi* bacteria DNA. The sensitivity test was started by performing a multilevel dilution six times on pure isolates of *Salmonella typhi* bacteria. Dilution uses 10 µL of pure bacteria isolate, and 40 µL of Nuclease Free Water (NFW) is added, then 10 µL of dilution results are taken to another dilution up to six dilutions. After obtaining the dilution results, each dilution was taken at 5 µL to be tested by real-time PCR (Mic qPCR Cycler Bio Molecular Systems).

3. Results

3.1. Primer of the *pipB* Design

F5'-GGGAGTGGAGTAGGGGTATG-F3' and R5'-AAGCAAACCTGACTCACGCA-R3' are the designed *pipB* primer. The primer length is 20 base pairs for each *pipB* Forward and *pipB* Reverse primer with an amplicon length of 196 bp. The *pipB* primer pairs

(*pipB*-F and *pipB*-R) were selected based on amplicon length, primer length, %GC, and melting temperature (Tm). The *in-silico* data of the designed *pipB* primer can be seen in Table 2, and the *pipB* primer nucleotide sequence based on the NCBI website database is shown in Figure 1.

3.2. Inoculation of *Salmonella typhi* Bacteria

The culture of *Salmonella typhi* bacteria incubated for 24 hours at 37°C (overnight culture) on Salmonella-Shigella Agar media produces a single colony that is black in the center (Figure 2). Furthermore, the results of bacteria growth on liquid media are indicated by changes in turbidity. Turbidity is shown by measuring Optical Density at a wavelength of 600 nm (OD_{600}) using a UV-Vis spectrophotometer to measure the concentration of pure bacteria cultured on liquid media. The inoculation process with Luria-Bertani broth liquid media produced an OD_{600} value of 1.834, indicating that the inoculation process was successful, and the bacteria grew on the Luria-Bertani broth liquid media.

3.3. *Salmonella typhi* DNA Extraction

Qualitative test results of *Salmonella typhi* bacteria DNA isolates showed that *Salmonella typhi* bacteria had been successfully isolated because the bands formed in lanes 2 to 5 appeared above 10,000 bp based on the marker (Figure 3). In the quantitative test, the purity and concentration of DNA were measured using a NanoDrop Spectrophotometer at a wavelength of A260/A280. The resulting concentrations (nucleic acid) were 191 ng/µL with a purity of 1.910 (elution 1) and 31 ng/µL with a purity of 1.330 (elution 2).

3.4. Optimization of the Annealing Temperature of the *pipB* Primer

The temperature range used in optimizing the annealing temperature in this study was 54-62°C using Gradient PCR. Based on the results obtained (Figure 4), the primer amplified *pipB* gene fragments at all temperature ranges. Based on the electrophoresis results, it can be seen that there is no non-specific amplification or miss-priming because only one band is formed at an amplicons size of about 196 bp (compared to DNA marker), where in-silico, the *pipB* primer amplicons length is 196 bp.

3.5. Confirmation Test of *Salmonella typhi*

Based on the amplification and melting curve of the confirmation test results in Figure 5, *pipB* gene primer recognized *Salmonella typhi* bacteria DNA on cycles 12.93 and 13.10 (Duplo), while NTC appeared at cycle 31.08. The melting curve shows that the pure culture of *Salmonella typhi* with *pipB* primer produces one peak at 84.05°C and 84.20°C (Duplo) with an isolate

Table 2. *In silico* data primer, the *pipB* gene *Salmonella typhi*

Primer	Sequence	Tm (°C)	%GC	Self-dimer (kcal/mol)	Hairpin (kcal/mol)	Cross dimer (kcal/mol)	Amplicon length	Primer length
pipB-F	F5'-GGGAGTGGAGTAGGGGTATG-F3'	55.79	60.0	-	-	4.52	196 bp	20
pipB-R	R5'-AAGCAAACCTGACTCACGCA-R3'	59.00	50.0	-	-			20

CTAAAATATTGGATGGGGGAAAAGCGTTTTATCATTGTAATCCGGGAGTGGAGTAGGGGTATGTTGACTA
CCTGTCAGATCGGCTCCTGTTAATGCTTTCGCTAAAGTTAGAGCTATCTTATCTAATTTTGCACCATTTA
GTTTGGTGTCAGTTAAGTCTGAGCCGAATAGAATTGCAGCGGTTAAGTTTACACCGGACATGTTAGCGCA
AGTCAGGTCTGCGTGAGTCAGGTTTGCTTTAGTCATGTTTGAACCCATTAGATTTACAGCGTGTAGATTT
GCACAACACAAATTTGCACCGACTAAATTTACATTAGATAGATTTGTTTCACGGAAATCTGCATTAGAAG
CATCTATGTCTGACAAATCTTCTCCCTGGAAATCTTTATGAGCTAGGTTTACTCCGCGCAGGTTCAGAAA
ACCATCCTCGGTTATAAGTGAATCAGGCTGTTGTATATTATGTTTACTCATCAGTTTTAACATTCGACAT
ACATTCCAAAATCTCTCACTATCAACTTTATCCGTTACAGTTTTTCCATTTTTGTTCACTTCAATAGTGA
CCATGGGATCCGTATTTTCGGATGCTGCTCCACATGACAGACATATAGTACACCCGTTGACATCCTCCAG
AAATATTTTATTACCATCGAAGAAAGCATCTTTATTTACATATAATAATGATGTGGTCAGTTTTCCAATT
ACCTCCCAAAAGCATCTTTCATTGCTTCTTCTTACTCCACCACAGGTAAAAAAATTGACAAACCATTCCA
GTATACCGCGTGGTGAAGTTGCACTTTTCATTGCTTCTTTCGTACCGGTACCGGCCGCATGCAAATATCT
TAATATATTTTCTGGGGACGCGTTAGTTATTGGCAT

Figure 1. Nucleotide sequence of *pipB* primer measuring 876 bp in the 1086906-1087781 region. *pipB* primer forward (yellow); *pipB* primer reverse (green); *pipB* primer amplicon (blue)

Figure 2. Colonies of *Salmonella typhi* on Salmonella-Shigella Agar media

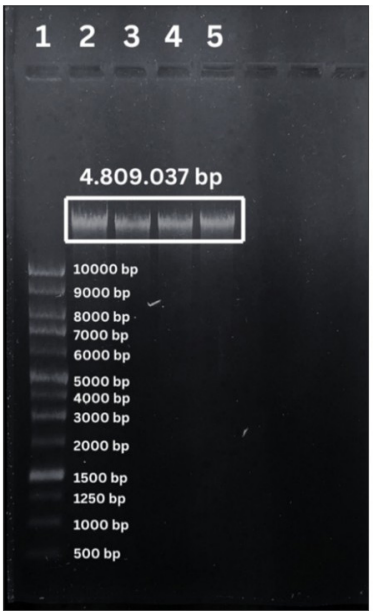


Figure 3. Characterization of *S. typhi* isolates on agarose gel electrophoresis. (1) 1 Kb DNA Ladder; (2 and 3) *S. typhi* DNA elution 1; (4 and 5) *S. typhi* DNA elution 2

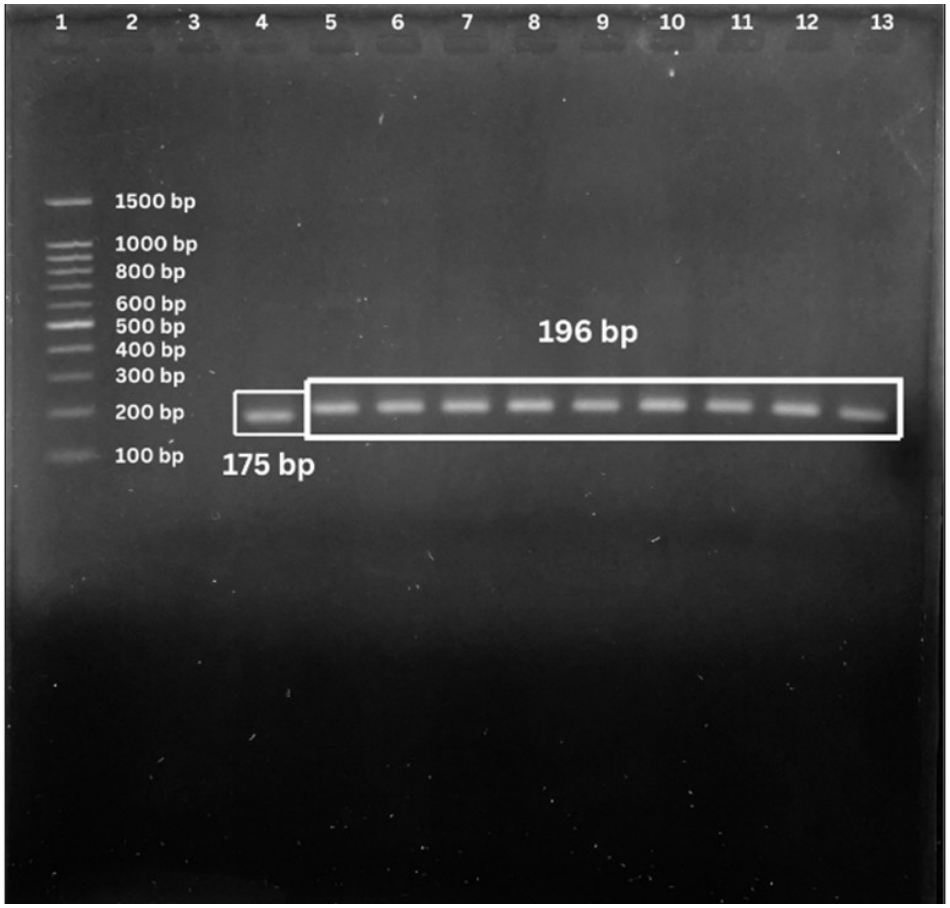


Figure 4. Results of optimation annealing temperature of *pipB* primer. (1) DNA ladder 1,000 bp; (2) non-template control (NTC); (3) NFW+MM; (4) codY *Bacillus subtilis* 175 bp as positive control; DNA fragment of annealing temperature (5) 54°C; (6) 55°C; (7) 56°C; (8) 57°C; (9) 58°C; (10) 59°C; (11) 60°C; (12) 61°C; (13) 62°C

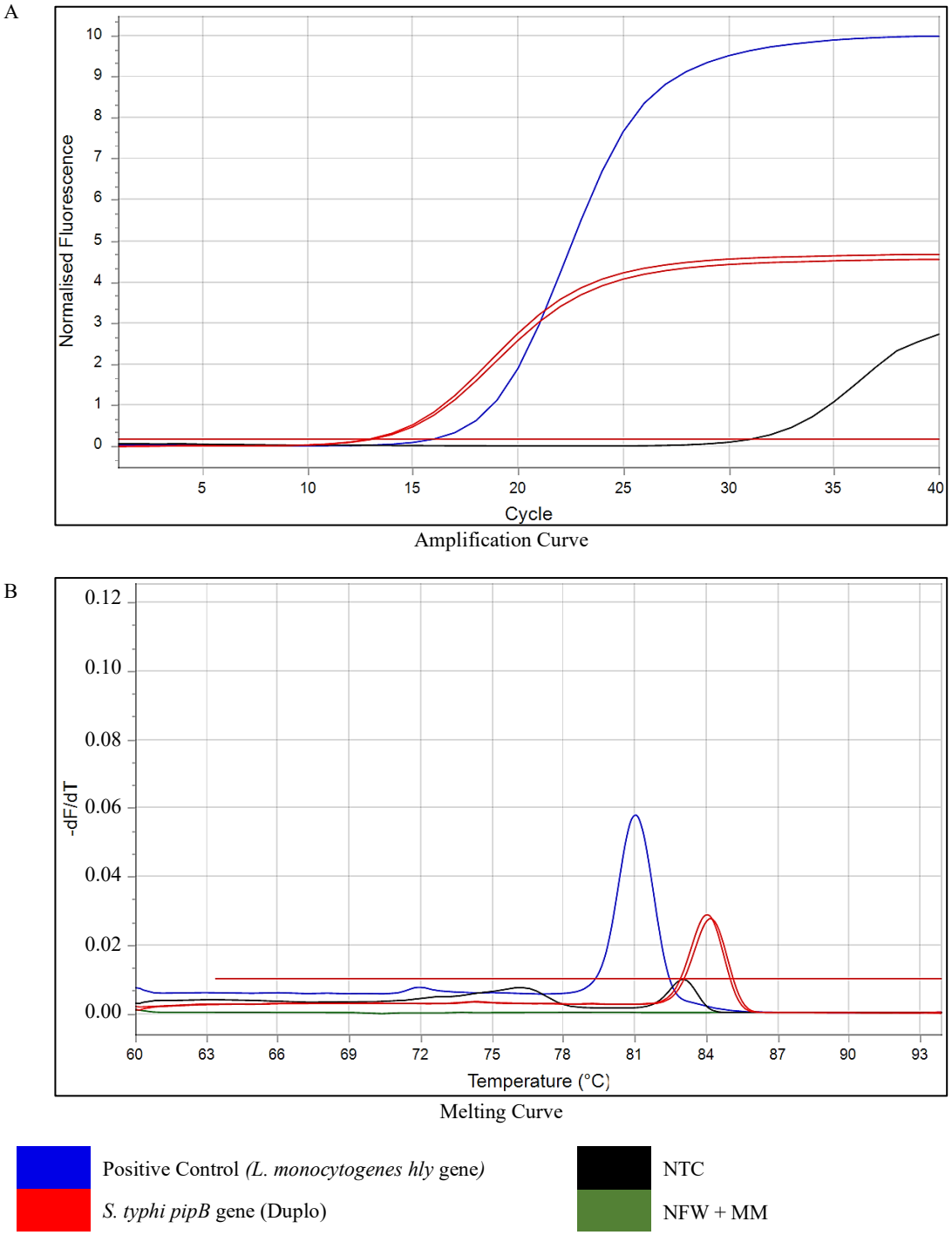


Figure 5. (A) Amplification curve of *S. typhi* the *pipB* primer confirmation test by real-time PCR, (B) melting curve of *S. typhi* the *pipB* primer confirmation test by real-time PCR

concentration of 10 ng/µL of pure culture DNA isolate and a primer concentration of 2 pmol.

3.6. Specificity and Sensitivity Test

Results of the *pipB* gene primer specificity test based on the cycle threshold (Ct) and melting temperature (Tm) values in Figure 6 and Table 3. The amplification curve on *Salmonella typhi* as a positive control obtained Ct values of 12.24 and 21.30 (Duplo). At the same time, the other nine non-target bacteria, such as *K. pneumoniae, P. aeruginosa, C. sakazakii, L. monocytogenes, E. coli, S. flexneri, Y. enterocolitica, E.*

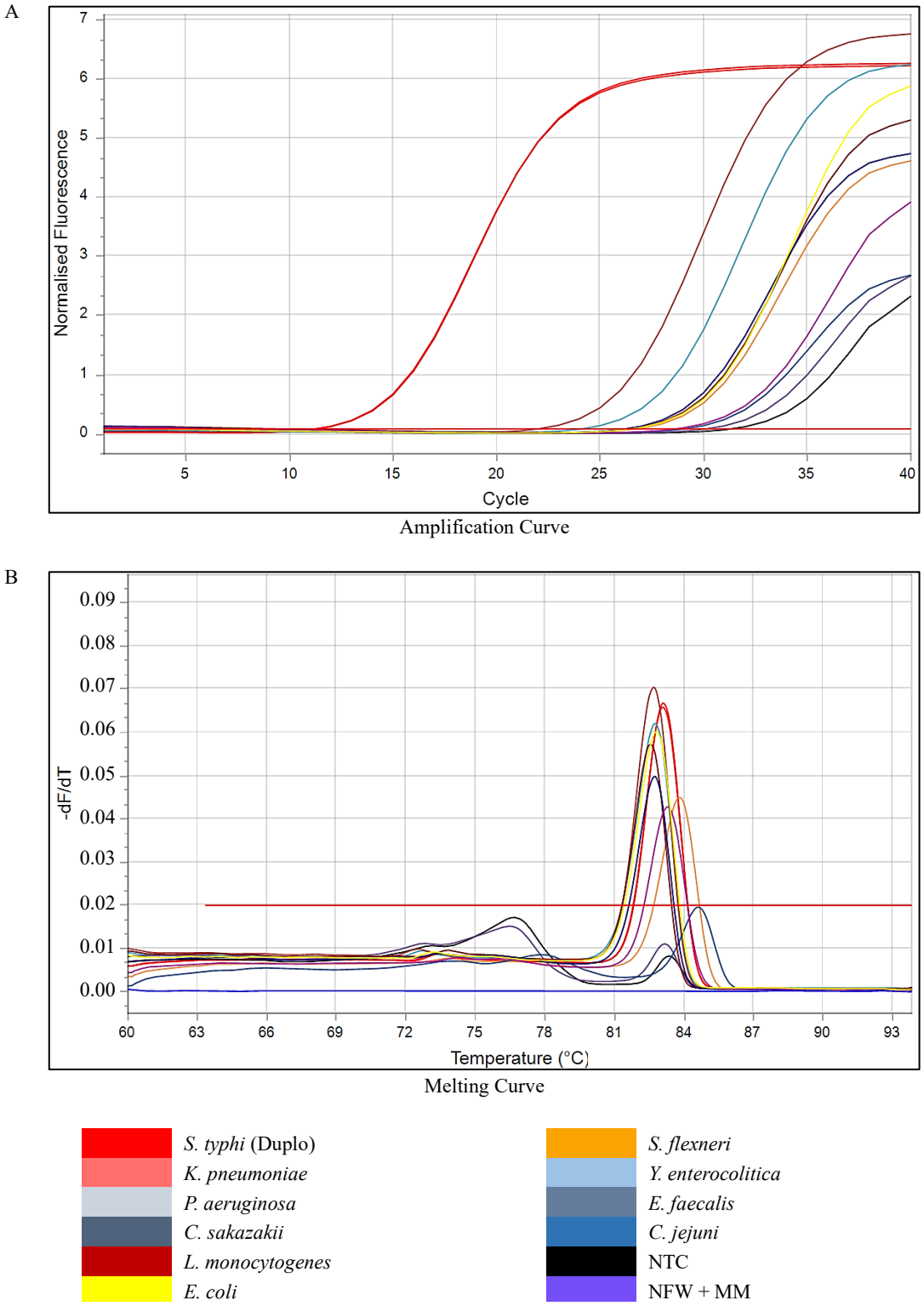


Figure 6. (A) Amplification curve of *S. typhi* the *pipB* primer specificity test by real-time PCR, (B) melting curve of *S. typhi* the *pipB* primer specificity test by real-time PCR

faecalis, and *C. jejuni* had Ct values ranging from 23 to 30. The amplification curve shows that the primer can distinguish between target and non-target bacteria because the appearance of nine non-target bacteria has a Ct of more than ten cycles, as well as NTC on Ct 30.31. The melting curve shows that each bacteria

Table 3. *Salmonella typhi pipB* primer specificity test results

Color	Samples	Ct	Tm (°C)
	S. typhi (Duplo)	12.24	83.11
		12.30	83.13
	K. pneumoniae	23.01	82.70
	P. aeruginosa	24.10	82.76
	C. sakazakii	26.22	82.77
	L. monocytogenes	26.42	82.57
	E. coli	26.48	82.82
	S. flexneri	26.72	83.82
	Y. enterocolitica	28.61	83.31
	E. faecalis	29.07	-
	C. jejuni	30.11	-
	NTC	31.30	-
	NFW + MM	-	-

has a specific Tm influenced by the sequence of the amplicons formed.

The results of the sensitivity test data are in Table 4 shows that the smaller the DNA concentration, the greater the Ct value. The lowest concentration is 0.0032 ng/µL with a Ct 25.23. The main result of the sensitivity test is the standard curve shown in Figure 7, which will determine the Limit of Detection (LoD) value of the *pipB* primer in detecting *Salmonella typhi* DNA. Based on the standard curve of the sensitivity test results, the line equation y = -3.88x + 15.47 was obtained, where y is the Ct value and x is the log concentration of sample bacteria. The coefficient of determination (R^2), which shows the accuracy of the data, produces a value of 0.9998 and an efficiency value of 0.81. The LoD value of 5.78×10^2 CFU is equivalent to 3.2 pg/µL of DNA concentration with a cut-off value of 33.

4. Discussion

Effective efforts are needed in dealing with victims of food poisoning cases caused by pathogenic bacteria, namely by designing detection methods that are very fast, specific, sensitive, and accurate in detecting pathogenic bacteria that cause food poisoning. One of the rapid and sensitive analysis methods in detecting pathogenic bacteria, especially *Salmonella typhi*, is the real-time Polymerase Chain Reaction method (Hein *et al.* 2006; Teh *et al.* 2021). In this study, the real-time PCR method was used to detect *Salmonella typhi* bacteria by targeting the *pipB* gene, one of the pathogenic genes in *Salmonella typhi* that can cause food poisoning. In previous research (Nurjayadi *et al.* 2019), rapid detection was carried out with the real-time PCR method against *Salmonella typhi* bacteria by targeting the *fimC* gene in egg samples.

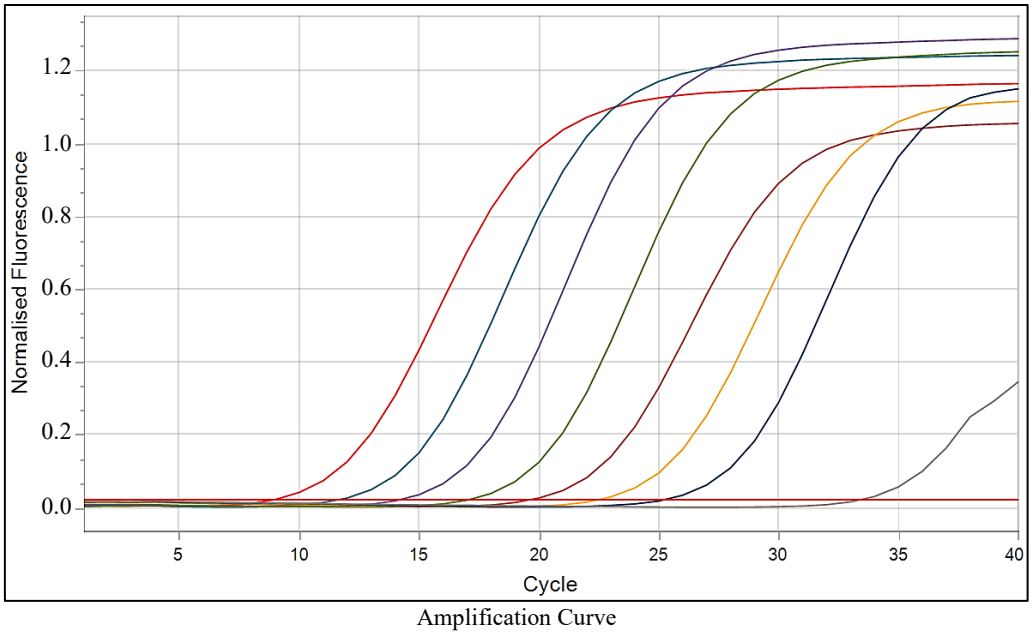
Table 4. *S. typhi* the *pipB* primer sensitivity test results

Color	Samples	Ct
	S. typhi 50 ng	8.94
	S. typhi 10 ng	11.62
	S. typhi 2 ng	14.18
	S. typhi 0.4 ng	17.07
	S. typhi 0.08 ng	19.62
	S. typhi 0.016 ng	22.45
	S. typhi 0.0032 ng	25.23
	NTC	33.40

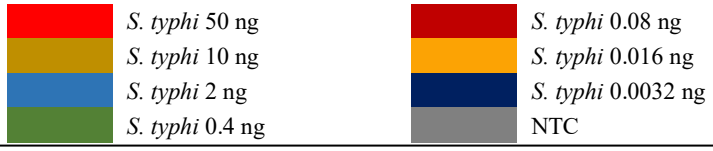
This study used the primer *Salmonella typhi pipB* gene with the design of *pipB*-F (forward primer) and *pipB*-R (reverse primer), as shown in Table 2. Figure 1, shows that the primer obtained produced an amplicon of 196 nucleotide base pairs (bp). *Salmonella typhi* bacteria DNA isolates obtained were then tested qualitatively with 0.7% agarose gel electrophoresis using Green Safe as fluorescent and visualized using a UV Transilluminator. Based on Figure 3, a bright DNA band from a pure culture of *Salmonella typhi* DNA isolate in lanes 2 to 5 appears in a position higher than the marker size of 10,000 bp and can be estimated that the results obtained correspond to the size of the entire genome sequence of *Salmonella typhi* strain CT18 which is 4,809,037 bp that will be validated the presence of *Salmonella typhi* DNA in isolate by real-time PCR method. DNA concentration and purity are shown in Table 5. According to the data obtained, one of the *Salmonella typhi* bacteria isolates has met the requirements, namely isolate elution 1. Because DNA isolates in elution 1 have purity in the range of 1.8-2.0. If the purity value is below 1.8, it indicates protein contamination in the isolate, while if the purity value is above 2.0, it indicates RNA contamination. The good quality purity value will have an A260/A280 ratio of 1.8-2.0 (Dewanata & Mushlih 2021).

The results of the confirmation test are shown in Figure 5A and B. In this study, positive control is used to verify that all reagents work. The negative controls used were Non-Template Control (NTC) and NFW (NFW+MM), and this reaction verifies whether there is a contamination reaction and the absence of primer-dimer formation. Based on the results of the confirmation test, it was found that the *pipB* gene primer that had been designed amplified the *pipB* gene fragment as the target region for detecting Salmonella typhi bacteria. The *pipB* primer can detect the presence of *Salmonella typhi* DNA with a concentration of 10 ng/µL at a cycle (Ct) of 12.93 and 13.10 (Duplo) with Tm values of 84.05°C and 84.20°C (Duplo). In the results,

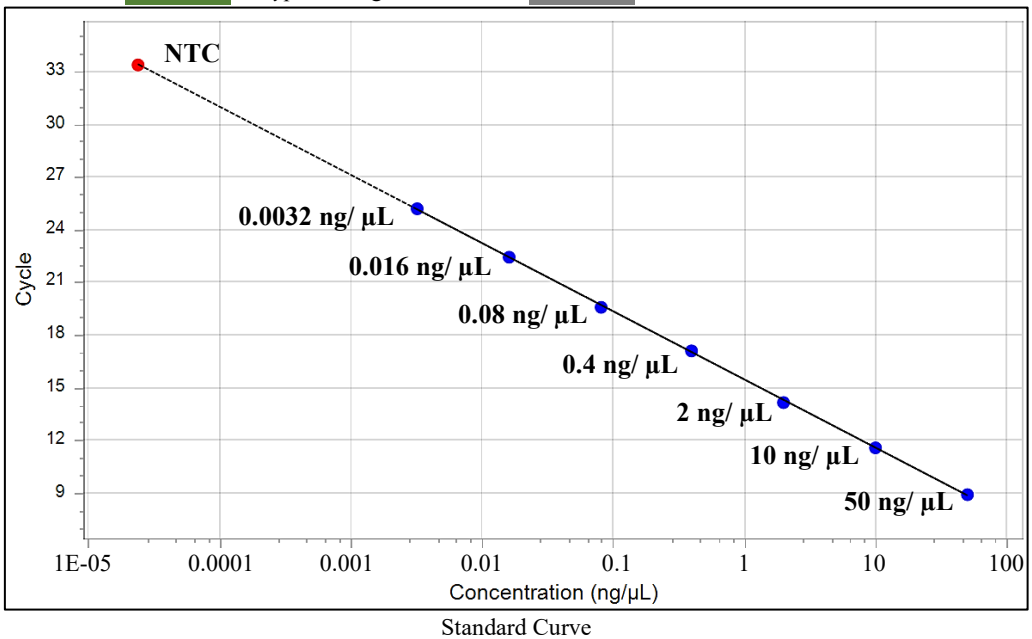
A



Amplification Curve



B



Standard Curve

Equation : y = -3.88x + 15.47
Efficiency : 0.81
R^2 : 0.9998

Figure 7. (A) Amplification curve of *S. typhi* the *pipB* primer sensitivity test by real-time PCR, (B) standard curve of *S. typhi* the *pipB* primer sensitivity test by real-time PCR

Table 5. Concentration and purity *S. typhi* DNA isolate

Samples	Concentration (nucleic acid) ng/µL	Purity (A260/A280)
S. typhi elution 1	191	1.910
S. typhi elution 2	31	1.330

NTC, as a negative control, still formed a sigmoid line on the amplification curve. The appearance of Ct on NTC is indicated by the formation of dimers on the primer. It is not a contamination because the NFW+MM negative control does not have the appearance of Ct, so it is indicated that the appearance of Ct on NTC is due to the presence of dimers. NTC appeared at cycle 31.08, and the amplification result was considered non-target because the cycle distance difference was more than ten cycles against the target bacteria *Salmonella typhi* (Dorak 2007). Analysis based on the difference and average results of the Ct and Tm value from the confirmation test to the specificity test with the real-time PCR method, *pipB* primer can amplify *Salmonella typhi* bacteria DNA at Ct 12.47±0.6 with a Tm value of 83.62°C±0.6.

In previous research conducted by (Nurjayadi *et al.* 2019), *Salmonella typhi fimC* gene primers were only tested with *Shigella flexneri* and *Escherichia coli* to test the level of primer specificity. However, in this study, pipB primers were tested with nine other bacteria that cause food poisoning to see the level of specificity of *pipB* primer in recognizing *Salmonella typhi* bacteria as target bacteria. Based on the amplification and melting curve of the specificity test in Figure 6A and B, *pipB* primer has a good level of specificity because it can distinguish between target bacteria and other non-target bacteria, which are also bacteria that cause food poisoning based on the Ct and Tm values. Sensitivity testing was done by multistage dilution of seven stages on *Salmonella typhi* DNA samples with concentrations determined for the sensitivity test. Based on the standard curve in the sensitivity test in Figure 7B, the standard curve analysis resulted in the equation y = -3.88x + 15.47 on *pipB* primer with a primer concentration of 2 pmol, with y representing the Ct value and x representing the base 10 logarithm of the DNA concentration in ng/µL units. Based on the line equation obtained, the coefficient of determination (R^2) was 0.9998. The R^2 value obtained in this study has met the quality standards that a good R^2 value is equal to or greater than 0.98 (Chicco *et al.* 2021). In this study, the LoD value of *pipB* primer was 5.78 × 10^2 CFU at cycle 25.23 with a cut-off value of 33, and based on the calculation of the relationship between DNA concentration and bacteria concentration, it was found that *pipB* primer can detect *Salmonella typhi* bacteria up to a concentration of 3.2 pg/µL.

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