

Evaluation of primer detection capabilities of *fimC* *Salmonella typhi* using real time PCR for rapid detection of bacteria causes of food poisoning

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Abstract. Food poisoning is a disease caused by bacterial, viral or parasitic infections, which contaminate food. The purpose of this study was to obtain information about the detection of the primer pair of *fimC* *Salmonella typhi* genes using the Real-time PCR methods for the rapid kit development. The evaluation of the primer ability is determined by the accumulation of fluorescence signal from the amplification curve connecting the number of Cycle threshold (Ct) to the intensity of the amplicon signal that can reach the threshold line. The results showed that the primer *fimC* gene successfully amplifies the *S. typhi* DNA target fragment on Ct 14.783. In addition, the Ct data from this study also informed that the primer sensitivity of the *fimC* gene against the target *S. typhi* bacteria gave a minimum detection rate of 4,528 pg/μL, and the primer specificity evaluation of that primer to non-targeted bacteria *Shigella dysenteriae* gave Ct 27,949. Based on the data it can be concluded that *fimC* *S. typhi* primers gene can be used as sensitive and fast detection devices, but still require improvement in the specificity.

1. Introduction

Food poisoning is a disease that caused by bacterial, viral, parasitic, or toxic infections from germs that contaminate food [1]. Many cases of poisoning occur within the community caused by pathogenic microbe lead required throughout the development of pathogen detection methods that fast, accurate, and sensitive to pathogens. The real time Polymerase Chain Reaction method can be an alternative method to detect foodborne pathogen bacteria; one of the bacteria is *Salmonella typhi* [2-5]. The real-time PCR method declared to have a high degree of sensitivity and specificity compared with conventional PCR methods and requires faster time compared to traditional methods or culture methods. [6-8].

Methods based on genomics develop rapidly since molecular biology was found, especially PCR in examination of pathogenic bacteria. One uses Taq DNA polymerase 5'nuclease to hydrolyze fluorogenic probes to monitor DNA target amplification. However, the application requires the availability of primers and very suitable probes and cannot always be easily applied. The use of double-stranded DNA dyes SYBR Green 1 is easier without requiring a probe associated with fluorescent molecules [9-10].



Previous research has managed to design the primer pair of *fimC* genes for *Salmonella typhi* bacteria. Primer pair of *fimC* genes have successfully amplified DNA of *Salmonella typhi* bacteria at 95 base pair (bp) by conventional PCR method [11]. However, the sensitivity and specificity of the primer pair to bacteria DNA that use this unknown. Sensitivity testing seen from the lowest concentrations of genomic DNA that can still produce DNA tape appropriate for the amplicon length and evident in gel electrophoresis. While the specificity is the ability of a primer pair that only recognizes one genome. Therefore, this study aims to test the sensitivity and specificity of the primer pair of *fimC* genes for *Salmonella typhi* bacteria using real-time PCR method.

2. Methods

Material has been used in this research consist of GeneJET isolation kit Genomic DNA purification (Digestion Solution, Lysis Solution, RNase A Solution, Proteinase K Solution, Wash Buffer, and Elution Buffer) (Thermo Scientific); 2x Q-PCR Master Mix SYBR, ROX (SMOBIO); primer gene *fimC* *S. typhi* (Macrogen Inc); Nuclease Free Water (Qiagen); pure cultures of *Salmonella typhi* (UI Microbiology Lab); Luria Bertani (Deben Diagnostic, Ltd.) media, and Salmonella Shigella Agar (Deben Diagnostic, Ltd). Preparation stage includes: culturing *Salmonella typhi* bacteria on selective and enriched media; DNA genomic isolation of *Salmonella typhi* bacteria, followed by amplification stage using real time PCR 7500 FAST (Applied Biosystem) instrument. Primer ability tests included (1) primer confirmation test of the *fimC* gene against the target bacterial DNA isolated (2) sensitivity test with dilution of the concentration of DNA isolates, and (3) the specificity test by cross-examining the specific primer gene to the non-target bacteria. Stage of making the reaction mixture follows the standard protocol from the master mix that used; the total volume in each reaction is 20 μ L, consist of 10 μ L master mix SYBR Green 1, 1 μ L forward primer (10 μ M), 1 μ L reversed Primer (10 μ M) specific bacteria, 2 μ L bacterial DNA isolated, and 6 μ L Nuclease Free Water solvent. The amplification process was carried out for 40 cycles with pre-denaturation stage at 95 $^{\circ}$ C for 3 minutes, denaturation stage at 95 $^{\circ}$ C for 10 seconds, annealing stage at 60 $^{\circ}$ C for 30 seconds, and extension stage at 72 $^{\circ}$ C during 30 seconds.

3. Results and discussion

The results from the primer confirmation test of the *fimC* *Salmonella typhi* gene in pure culture bacteria were successfully amplified as shown in figure 1. The sample test of bacterial DNA isolated samples were duplicated, and the NTC was used as negative control. Based on the results from the amplification curve indicate the primer *fimC* gene successfully amplify samples of genomic DNA isolates *Salmonella typhi* with the formation of red and yellow sigmoid graphs that made it through the baseline threshold by forming the intersection point of the Cycle threshold (Ct) 14.783 and 14.923 with the intensity of fluorescence signal reached for 0.398786 is indicated by a horizontal yellow line. No Template Control (NTC) shown by sigmoid graph, amplified on Cycle threshold (Ct) 32.631. Based on threshold cycle (Ct) literature, good NTC should be above the 35 cycle indicating that no non-targeted DNA is involved in amplification. However, the NTC threshold cycle that has a distant vulnerability exceeding 10 threshold cycles of the final threshold cycle reached are still within the limit of tolerance for the amplification of non-targeted DNA contamination [6].

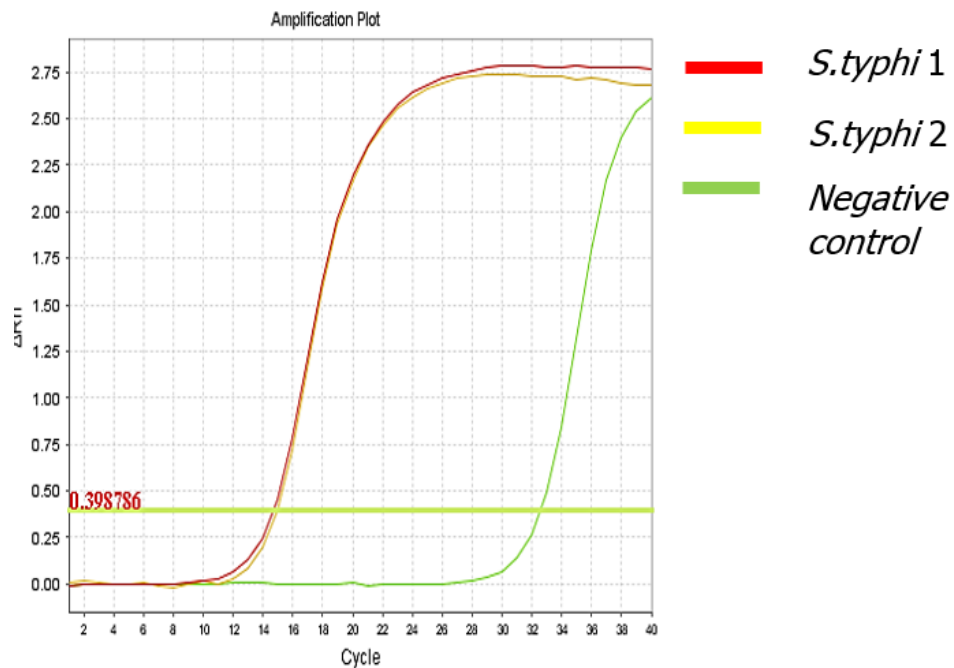


Figure 1. The amplification curve of the confirmation test of Primary Fim-C *Salmonella typhi* gene.

Primer confirmation test is also done by looking at Melt Curve Analysis (MCA) data. Results of the melting point curve yield one peak with value T_m 83.01°C. The yield of the melting point curve indicates that the test does not occur mispriming means that the primer used does not amplify the gene other than the target gene shown by the formation of a peak [6-7, 16-17], shown in figure 2.

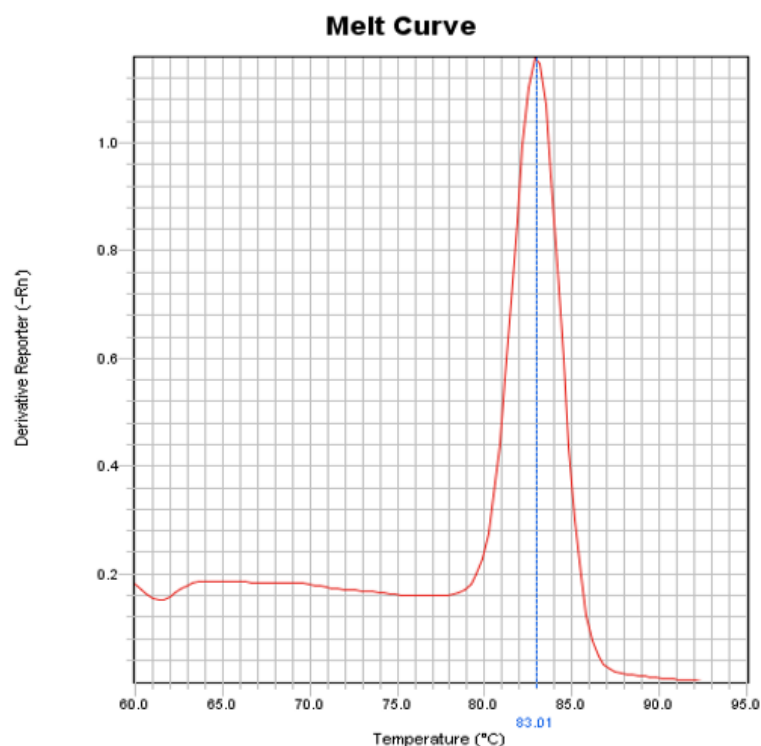


Figure 2. The peak melting curve of confirmation test of Primary Fim-C *Salmonella typhi* gene.

The sensitivity test results were performed by DNA sample stratified dilution up to the specified concentration. The results of the information indicated by the amplification curve and the standard curve with good efficiency, indicating the number of amplified DNA templates multiplying in each cycle at each dilution concentration. This is evident to the significant increasing threshold cycle (Ct) shows by the sigmoid graph formed in figure 3.

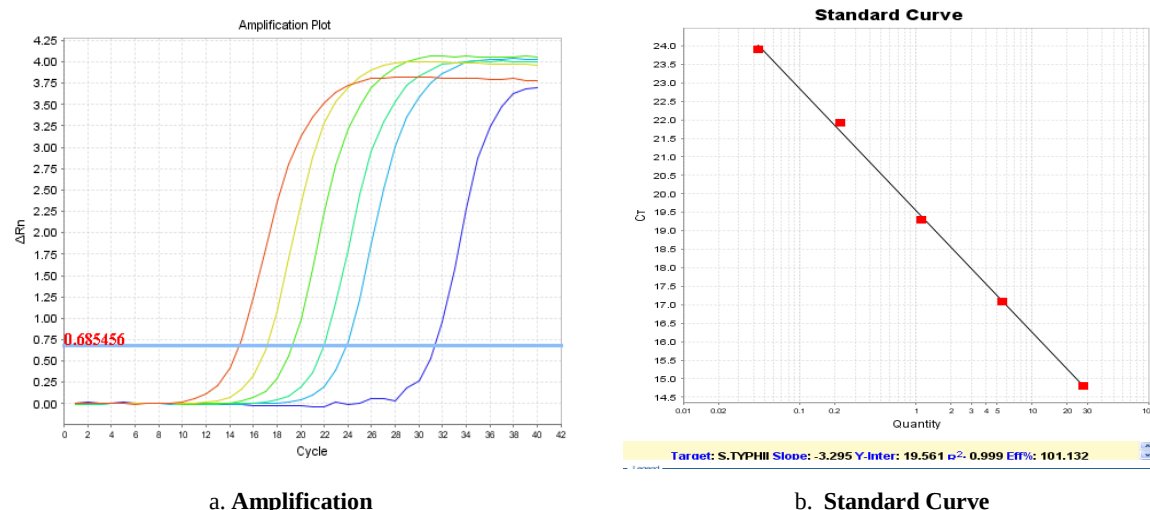


Figure 3. Curve of amplification and standard curve of sensitivity test results of primer *fim-C* genes *Salmonella typhi*.

The results of the *Salmonella typhi* amplification curve at the lowest concentration of the DNA template can still be amplified by the bacteria-specific primer gene and reach the threshold line with a Ct value of Ct 23.90 at the lowest concentration of 4,528 pg/ μ L, shown in table 1.

Based on figure 4, the results from the amplification of the specificity test of the *fim-C* primer gene *Salmonella typhi* shows primer gene *fim-C* *Salmonella typhi* can recognize its own DNA as the target DNA of *Salmonella typhi* indicated by Ct value of 14.770 while the primer of the *fim-C* genes *Salmonella typhi* is considered unable to recognize the DNA of *Shigella dysenteriae* as non-target DNA despite amplification in Ct 27 949. This is because the difference in *Shigella* DNA amplification is more than 10 cycles compared to the *Salmonella typhi* *fim-C* genes. Ct of a positive sample is lower 10 cycles or more of negative control Ct or NTC, then the sample is considered negative [6, 18], so it can be concluded that the *Salmonella typhi*-specific *fim-C* gene primers are specific against *Salmonella typhi* bacteria on the average Ct of the 14th cycle, although it still requires development.

Table 1. Primer sensitivity test results of the *fim-C* gene *Salmonella typhi*.

Primer	Concentration DNA (pg/ μ L)	Line sigmoid	Thresholds Cycle(Ct)
gene <i>fim-C</i> <i>Salmonella typhi</i>	2783		14.80
	556.6		17.09
	113.2		19.29
	22.64		21.93
	4.528		23.90
	NTC		31.36
Slope : -3. 295; R^2 : 0.999; Eff %: 101.132 %; y-Int : 19.156			

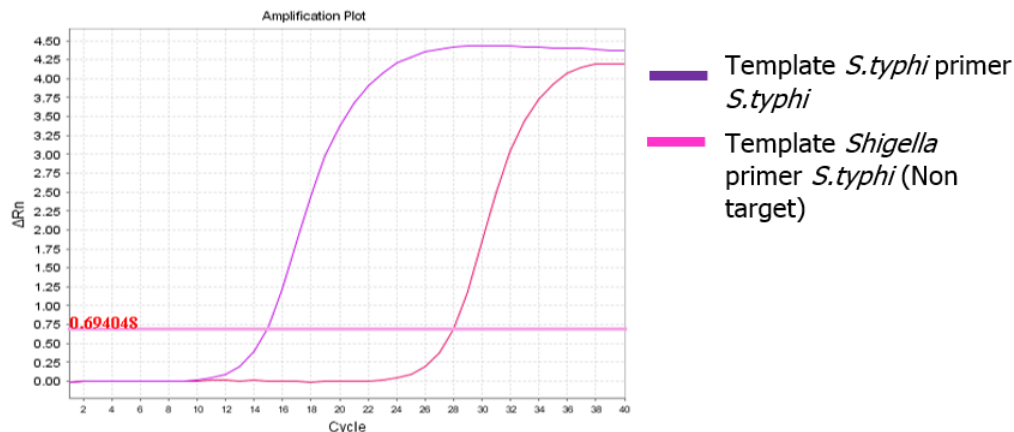


Figure 4. Specificity test amplification curve Primer genes fimC *Salmonella typhi* against target DNA and non-target.

4. Conclusion

The results from the primer evaluation using the real-time PCR method concluded that the *Salmonella typhi* fimC primer genes could serve as sensitive and rapid detection devices, but still requires improvement in the specificity of the fimC *S.typhi* primer gene, although the specificity of the fimC primer gene against the non-target bacteria gives a Ct value different.

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