In Silico Study of Developing a Method for Detecting Pathogenic Bacteria in Refillable Drinking Water Samples

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Abstract. One of the parameters of quality of drinking water that is suitable for consumption is not contaminated by Escherichia coli, Salmonella and Shigella sp. Beside microbiological tests, water quality testing can be carried out molecularly only in few hours, using PCR (Polymerase Chain Reactions) technique. Although PCR is a basic molecular technique, various detection methods can be developed from it. The key to success in making a PCR-based method is inseparable from bioinformatics studies when designing primers that are specific to pathogen target DNA. The purpose of this research was to design pathogen-specific primers and do in silico study of PCR using bioinformatics software to get better planning in developing detection method. DNA sequence templates of E. coli, Salmonella and Shigella were downloaded from NCBI and multiple-aligned using Geneious Prime bioinformatic software. Primers were designed according to conserved region of these pathogens. The primers specificity was checked using Primer BLAST tools in NCBI. The result of this study was a pair of primers that amplify 825 bp fragment of 16S rRNA sequence specific to E. coli, Salmonella and Shigella.

1. Introduction
About 70 percent of human body is made up of water. Body fluid will lose constantly as we sweat and urinate. Therefore, if there is a lack of water then the body will not work perfectly. Each person's drinking water needs vary depending on gender and age. However, generally the body needs about 8 glasses of drinking water per day. The existence of Refillable Drinking Water Depot (Depot Air Minum Isi Ulang/DAMIU) helps people in meeting the needs of drinking water because of its practicality and affordable price. The use of drinking water sources from the Refillable Drinking Water Depot should always be considered for its quality and feasibility. Factors that can affect the quality of refillable drinking water, namely: raw water source, depot condition, operator cleanliness and handling of containers before being filled with refillable drinking water produced [1, 2, 3, 4].

Refillable drinking water treatment is processed through three stages: filtration, disinfection and filling. Poor quality drinking water will have an impact on health. Water can be a living habitat for bacteria and a medium for the spread of certain diseases such as diarrhea. One of the parameters of the quality of drinking water is not contaminated by the bacteria Escherichia coli, Salmonella typhi and Shigella sp. [5, 6, 7].

In general, microbiological analysis of refilled drinking water using only conventional methods and has several drawbacks, such as the difficulty in growing bacteria because the number of microorganisms contained in refilled drinking water is usually very small and the process of
identifying pathogenic microorganisms in refillable drinking water takes a long time. Water analysis testing techniques in addition to microbiological tests can be done molecularly, one of which is PCR (Polymerase Chain Reaction) technique. PCR has been widely used to identify bacteria in a rapid time and require a small number of samples [8].

Although PCR is a basic molecular technique, various detection methods can be developed from it. The key to success in making a PCR-based method is inseparable from bioinformatics studies when designing primers that are specific to pathogen target DNA. The purpose of this research was to design pathogen-specific primers and do in silico study of PCR using bioinformatics software to get better planning in developing detection method.

2. Material and Methods

2.1. Primer Design

DNA sequence templates of *E. coli*, *Salmonella* and *Shigella* were downloaded from NCBI and multiple-aligned using Geneious Prime bioinformatic software (https://www.geneious.com). Primers were designed according to conserved region of these pathogens. The primers specificity was checked using Primer BLAST tools in NCBI.

2.2. In Silico PCR

Genome Compiler bioinformatic software was applied to conduct PCR simulation or in silico PCR (https://designer.genomecompiler.com/app). The DNA template and primer sequences were input to the tools of generate PCR product. This software also had gel electrophoresis simulations to visualize the DNA band of PCR product.

3. Results and Discussion

3.1. Primer Design

Specific primers of *E. coli*, *Salmonella* and *Shigella* were designed using bioinformatic software, Geneious Prime. The target gene was the gene that encodes 16S rRNA. In order to obtain a specific primer sequence, the sequence of genes 16S rRNA *E. coli*, *Salmonella* and *Shigella* downloaded from NCBI, aligned first with multiple alignment on Geneious Prime. Primers are designed in areas that have conserved sequences for each species so that they can amplify simultaneously in a single PCR (Figure 1).

![Figure 1. Specific primer design of E. coli, Salmonella and Shigella on multiple alignment results using Geneious](image-url)
According to the guideline of PCR primer designing, the primer must meet the ideal criteria to have nucleotide lengths ranging from 18-30 mer, have a GC composition of 50%, Tm between the forward and reverse primer has no difference of more than 2-3°C, and does not form a secondary structure (hairpin or self-dimer)\textsuperscript{[9,10]}. Therefore, these parameters are re-examined using Geneious Prime, as described in Table 1.

**Table 1.** Geneious Prime Output of Designed Primer Characteristic

| No. | Primer Characteristics | DNA Fold |
|-----|-----------------------|----------|
| 1   | Name: ESS-forward     | ![Sequence](image1) |
|     | Type: Primer Bind (primer_bind) (Created by primer3) |
|     | Length: 20            |
|     | Interval: 84 -> 103   |
|     | Mismatch Positions: =====CT==C========== |
|     | Mismatches: 3         |
|     | %GC: 50.0             |
|     | Hairpin Tm: 51.2      |
|     | Self-Dimer Tm: None   |
|     | Tm: 60.0              |
|     | Pair Dimer Tm: None   |
|     | Sequence: TTGCTCTTTTCGCTGACGAGT |
|     | Product Size: 825     |
| 2   | Name: ESS-reverse     | ![Sequence](image2) |
|     | Type: Primer Bind (primer_bind) (Created by primer3) |
|     | Length: 20            |
|     | Interval: 909 -> 890 (908 -> 889) |
|     | Mismatches: 0         |
|     | %GC: 50.0             |
|     | Hairpin Tm: None      |
|     | Self-Dimer Tm: 28.7   |
|     | Tm: 60.0              |
|     | Pair Dimer Tm: None   |
|     | Sequence: TTTAACCTTGCGGCCGTACT |
|     | Product Size: 825     |

Specificity of the designed primers were rechecked with Primer BLAST tools at NCBI website\textsuperscript{[11]}. NCBI Primer BLAST results (Figure 2) showed that the primer pair can amplify the 16S rRNA gene of *E. coli*, *Salmonella* and *Shigella* with a product size of 825 bp.
3.2. In Silico PCR

PCR in silico was performed for the primer pair with each sequence of genes 16S rRNA from *E. coli* (Figure 3), *Shigella dysentriae* (Figure 4), *Salmonella typhimurium* (Figure 5) using Genome Compiler software with simulated electrophoresis gel results.

![Figure 3. Genome Compiler in silico PCR result of ESS-Forward and ESS-Reverse using *E. coli* 16S rRNA sequence as DNA template](image)

**Figure 3.** Genome Compiler in silico PCR result of ESS-Forward and ESS-Reverse using *E. coli* 16S rRNA sequence as DNA template.
In silico PCR also called electronic PCR or virtual PCR because it amplified computationally using bioinformatic software. The result of in silico PCR for each DNA template were consistent with the result of Primer BLAST. The importance of doing in silico PCR before conducting experiments in the laboratory is to prevent errors in primer designing so that the primers anneal not specific to the target gene only \cite{12,13}.
4. Conclusion

A pair of primers were generated from this study that amplify simultaneously 825 bp fragment of E. coli, Salmonella and Shigella 16SrRNA gene.

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