Primer Designing For Molecular Detection Of Salmonella Spp Based On ParC Gene

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Abstract— It has been reported the use of QRDR genes that improve taxonomical resolution among member of Salmonella. Higher taxonomical resolution was reportedly known by using four genetic markers for QRDR region, namely gyrA, gyrB, parC, and parE genes, respectively (Amarantini and Satwika, 2015). In this study, polymerase chain reaction primers, based on parC gene of Indonesian indigenous Salmonella strains, were designed and used for further specific detection of Salmonella spp. ClustalX, MEGA6, and GeneDoc were used as tools for designing the new primer. Two sets of novel PCR primers, namely parChF and parChR have been developed. The primer pair demonstrated the ability to amplify the targeted gene region. To validate the assay, genomic DNA from Salmonella strains isolated from iced tea, cow’s milk, and goat’s milk were subjected to PCR. The new primer reported here have high resolution, demonstrating its potential for separating Salmonella spp.

Keywords: Salmonella sp, PCR, parC, primer designing

I. INTRODUCTION

Salmonella is known as a big group of bacteria with high diversity among them. Based on its serotypes, it is already realized a number of about 2,500 species. This group is now grouped into 5 species, i.e.: Salmonella arizonae (type strain: ATCC 13314), Salmonella choleraesuis corrig. (type strain: ATCC 13312), Salmonella enteritidis (type strain: ATCC 13076), Salmonella typhi (type strain: ATCC 19430) Salmonella typhimurium (type strain ATCC 13311) (Skerman et al., 1980). Later on it is found a new species, known as Salmonella subterranea (Shelobolina et al., 2004).

Salmonella enterica subsp. enterica serotype Typhi (Salmonella Typhi) is the only member of Salmonella with pathogenic property, causing typhoid fever for man; man is the only host for this bacteria. This fever is known to be infected via fecal-oral through contaminated food and water (Kothari et al., 2008).

Molecular detection is possible if one specific marker has been known. As the high molecular diversity is realized among Salmonella Typhi strains, it is important to find out molecular marker specific for it based on local isolates. It is then fundamental to isolate indigenous Salmonella Typhi from Indonesia. It has been reported the use of gyrB and QRDR genes that improve taxonomical resolution among member of Salmonella. The gyrB gene shown to be the best intraspecies genetic marker for strains belonging to Salmonella Typhi. It produces higher resolution compared to 16S rRNA, as more clades are produced with more variations (Amarantini and Satwika, 2014). Higher taxonomical resolution was reportedly known by using four genetic markers for QRDR region, namely gyrA, gyrB, parC, and parE genes, respectively, with the order of higher taxonomic resolution based on phylogenetic topological structure and similarity value as follow: parC, gyrB, parE, and gyrA (Amarantini and Satwika, 2015). Polyphasic taxonomic analysis revealed that parC is a potent candidate as molecular marker. It produces the best consistency compared to gyrA, gyrB, and parE genes as shown by its bootstrap value of 99 and 100 (1000 replicate) (Amarantini and Satwika, 2015).

Based on that finding, a research is done to improve molecular identification method by primer designing using conserved nucleotide sequences of parC from indigenous isolates to differentiate members of Salmonella. The resulting molecular marker (primer pair) was designed to improve specificity and sensitivity as Salmonella marker that could differentiate members of salmonellae with other closely related microbes.
II. MATERIAL AND METHODS

A. Bacterial cultures

Four indigenous isolate of Salmonella Typhi obtained from previous studies, denotes as BPE 122.4 CCA R*, BPE 127.1 MC R*, BPE 122.1 CCA*, dan RSK 5.1 SSA* were used (Amarantini et al., 2009; Amarantini et al., 2011; Amarantini et al., 2012; Amarantini and Budiarsno, 2013; Amarantini and Satwika, 2014). Salmonella Typhi NCTC 786 (PT. Biofarma) and Salmonella Typhi strain O (BLK Yogyakarta) were used as positive control.

B. Isolation of DNA

DNA was isolated by using standard phenol-chloroform-isoamyl alcohol method (Sambrook et al., 1989). Isolation was done according to the protocol written in the manual. Purified DNA was checked electrophoretically as a single band above 12 kb mark on the gel.

C. PCR and sequencing of parC gene

Bacterial DNA, including the control DNA were amplified using standard primer pair (parCF, 5’ ATgAgCgATATggCAgAgCg 3’ and parCR, 5’ TgACCgAgTTCgCTTAACAg 3’) (Ling et al., 2003) according to the Dream TaqTM Green PCR Mastermix (Fermentas) manual. A gradient thermocycler PeqSTAR 2X was used. The resulting amplicon of 412 bp was realized as a single band on 3% electrophoresis gel, visualized by mean of Major Science UV transluminator. PCR products were then purified by using QIAquick gel extraction kit (Qiagen) following the instruction from the manufacture, and continued by sequencing which was done by Macrogen, Korea.

D. Primer designing of parC gene

An in silico study was done for designing a new primer pair which was deduced from nucleotide sequences of parC gene of isolates obtained from Indonesia. Targetted gene sequences obtained were analysed by using CustalX2 (Larkin et al., 2007), realigned by MEGA5 (Tamura et al., 2011) and documented by GeneDoc (www.psc.edu/biomed/genedoc). The resulting conserved sequence was then compared to the database available online by running BLAST. Based on the conserved area obtained, it is used for designing primer pair by using Clone Manager. The newly designed primer was also used for in silico PCR and the resulting amplicon was then compared to the online database for creating a phylogenetic tree.

III. RESULT AND DISCUSSION

In silico study of the newly designed primer was described below. PCR product targetting parC gene of DNA from local Salmonella sp strains was sequenced, and then analyzed to checked its similarity. The resulting alignment could be seen in Fig. 1 below, showing the conserved area.

FIGURE 1. Multiple alignment of nucleotide sequences of parC gene of indigenous Salmonella sp by using Mega5 and visualized by GeneDoc showing the conserved area (Amarantini and Satwika, 2015).
Based on the conserved area obtained, a new primer pair was designed covering that area. Consideration must be taken in primer designing, especially those which are related to primer specificity and it should not give a mispriming product. The resulting primer pair was named parChF and parChR (sequence data not shown).

For checking the specificity of the new primer pair, the in silico study was done to classify Salmonella sp or other organism by comparing to the available online database on GenBank. The resulting phylogenetic tree is shown in Fig. 2 showing that Salmonella Typhi is separated into two clades, while E. coli which is traditionally known as a closely related species to Salmonella sp is recognized as an outgroup. This is reflecting the specificity of the new primer pair to identify and separating Salmonella sp strains.

![Alignment and phylogenetic tree of Salmonella sp based on parC gene generated from new primer pair deduced from conserved area of parC gene of indigenous Salmonella sp from Indonesia.](image)

**FIGURE 2.** Alignment and phylogenetic tree of Salmonella sp based on parC gene generated from new primer pair deduced from conserved area of parC gene of indigenous Salmonella sp from Indonesia.

As can be seen from the figure above, the newly designed primer produce a taxonomical resolution that could be separated all of the tested strains from E. coli ATCC 25922 used as negative control. These results are consistent with the results of in vitro study. In vitro assay of this new primer pair showed the single band of expected length of 315 bp, either for quinolone resistant strain (122.4R), or the sensitive one (122.1 CCAS), as well as for the type strain Salmonella Typhi NCTC 786. It could also be seen an amplicon for non-target organism, which is E. coli ATCC 25922 used as negative control. However, the PCR product is disappearing when the annealing temperature was set at a higher temperature, i.e. at 60-62°C (Amarantini and Satwika, 2015).

Based on that finding, it could be said that the new primer pair could specifically amplify target gene parC of Salmonella sp, and differentiate it with the closely related species like E. coli. The newly designed primer pair will be submitted to the GenBank so it could be used for rapid and sensitive detection of Salmonella sp based on its QRDR genes.
IV. CONCLUSION

The newly designed primer pair which was deduced based on conserved nucleotide sequence of indigenous Salmonella isolates was proven to be specific for members of Salmonella. It could be used for rapid and sensitive detection of Salmonella spp.

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REFERENCES


