Detection of *Salmonella* sp. in fisheries product using real-time PCR

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**Abstract.** *Salmonella* is pathogenic bacteria causing intestinal diseases or typhoid fever. Contamination of fisheries products by these bacteria could lead to a customer dissatisfaction and product recall. In this study, *Salmonella* contamination in 25 seafood and seafood products obtained from traditional and modern retailers were evaluated using real time PCR. Two primers were designed to amplify a 204 bp target gene specific to *Salmonella*. These primers were successfully amplified the target gene of *Salmonella typhimurium* (ATCC 25241). However, the melting curves of the product samples were found below the threshold Cycle (Ct) value, indicating that *Salmonella* bacteria contaminated none of the fish and fisheries products.

**Keywords**: food safety, fisheries products, real-time PCR, *Salmonella*

1. Introduction

*Salmonella* contamination on fish products is often reported. This contamination usually occurs due to improper handling (Amagliani *et al* 2012). *Salmonella* is a pathogenic bacteria causing gastrointestinal disease and typhoid fever, and in severe cases, causing death. This bacteria caused seafood-related outbreaks in European Union (EFSA 2014), the United States (Barret *et al* 2017) and other countries worldwide. In Indonesia, contamination of *Salmonella* on fish products is quite common. Recently, a published paper reported contamination of *Salmonella* on 93% of seafood products in Surabaya (Pramono *et al* 2019).

Conventional methods such as agar method or enzyme-linked immunosorbent assay are still commonly used to detect *Salmonella* contamination on seafood products in Indonesia. These methods, however, are time-consuming and unpractical. The agar method requires 4 days to detect positive contamination and needs another 3 days to identify the species of the bacteria. Moreover, this agar method is experiencing high false negative (Siala 2017). Real-time PCR for *Salmonella* detection is gaining more attention because of their sensitivity and rapid results (Kasturi and Drgon 2017, Siala *et al* 2017). Rakesh *et al* (2010) developed SYBR Green-based real-time PCR for detecting *Salmonella* on seafood products. This method could detect *Salmonella* in water as low as 2 CFU/mL. This research was aimed to detect *Salmonella* contamination on seafood products from traditional and modern markets in Bogor surrounding area using SYBR Green-based real-time PCR.
2. Materials and methods

Twenty-five seafood products were obtained from traditional and modern markets in Bogor surrounding areas. The samples covered various products including fresh seafood, traditional-processed seafood, and modern-processed seafood. *Salmonella typhimurium* (ATCC 25241) was used as positive control.

2.1. Isolation and quantification of DNA from *S. typhimurium* and seafood products.

The DNA of *S. typhimurium* was isolated using Qiamp DNA minikit following the manufacturer’s instruction. Meanwhile, the DNA from fresh seafood and seafood products was isolated using DNeasy Blood and Tissue kit and DNeasy Mericon Foodkit, respectively. The DNAs were quantified using NanoPhotometer (Implen p360, Munchen, German).

2.2. Real-time PCR for Salmonella detection.

Real-time PCR was run on QIAGEN’s real-time PCR cycler, the Rotor-Gene Q (Qiagene, Hilden, Germany). A forward (5′ ATC GCT GAC TTA TGC AAT CG 3′) and reverse primer (5′ CGG GTT GCG TTA TAG GTCTG 3′) were used to amplify ompC gene of *Salmonella*. A real-time PCR mixture consisting of 12.5 µL Qfast SYBR green mix, 2.5 µL of each primer (10uM), 5.5 µL RNase free water and 2 µL of DNA was put in the cycler. The real-time PCR was performed using two-step cycling method consisting of initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 54°C for 30 s and melt on green at 60-95°C.

3. Results and discussion

Twenty-five seafood products were obtained from markets in Bogor surrounding areas and their DNA was isolated. The DNA was successfully extracted from the seafood products with the highest concentration was 282 ng/µL, although DNA from six products had concentration below the detection limit of the Nanophotometer (<10 ng/µL) (table 1). Table 1 shows that high DNA concentration was obtained from fresh and less-processed seafood, while highly-processed seafood such as shrimp paste or otak-otak contained very low DNA content. Previous study on tuna muscle found that non-processed samples yielded high amount of DNA while canned muscle, spread, and pâté yielded the lowest amounts of DNA (Piskata et al 2017). It is known that processing methods affect DNA quantity and quality, and different extraction methods are required to extract the DNA. Processing procedures could lead to DNA degradation, meanwhile filling media in the processed seafood affect the efficiency of DNA extraction process (Sajali et al 2018).

*Salmonella* ompC gene was amplified using a set of primers resulting in a 204 bp long amplicon. The ompC gene is a highly conserved gene within *Salmonella* serotypes. This gene encodes a major outer membrane protein (OMP) of *Salmonella* that is highly expressed in both low and high osmolarity (Jha et al 2012). The primers successfully amplified the ompC gene of *Salmonella typhimurium* (ATCC 25241) resulting in a single melting curve peak at temperature between 85°C and 90°C (figure 1). Previously, the primer pair has been successfully used to detect different *Salmonella* serotypes in various sources. Alvarez et al (2004) carried out multiplex PCR assay to confirm *Salmonella* from 138 microbial strains isolated from veterinary, environmental, food, and clinical sources and found the primers specifically amplified ompC gene from all *Salmonella* strains. The utility of the primer pair was further confirmed by Ngan et al (2010).

Due to the utility of ompC as a pan-*Salmonella* indicator, the gene was used to detect *Salmonella* contamination on twenty-five seafood products in Bogor. The melting curves from real-time PCR for these twenty-five products are presented in figure 2. The curves were found below the positive threshold, indicating none of the products contaminated with *Salmonella*. In contrast, using conventional approach, Pramono et al (2019) found 93.10% of seafood and seafood products from...
Surabaya traditional market were contaminated by *Salmonella* bacteria, eight of those were antibiotic-resistant *Salmonella* serotype. *Salmonella* is a pathogenic bacteria commonly contaminating meat and poultry. However, *Salmonella* outbreaks due to seafood consumption are increasingly reported (Fernandes *et al* 2018). Seafood is not considered as the natural habitat of *Salmonella*. Contamination of these bacteria on seafood products is mainly due to poor handling processes, particularly the usage of contaminated water (Kumar *et al* 2015, Fernandes *et al* 2018).

| Sample code | Products               | Sampling time (ETC +7) | DNA concentration (ng/µl) |
|-------------|------------------------|------------------------|--------------------------|
| **Traditional markets** |                         |                        |                          |
| Fresh       |                        |                        |                          |
| 1           | Squid                  | Monday, 16-3-2015, 06.30 | 14.00                    |
| 2           | Indian mackarel        | Monday, 16-3-2015, 06.30 | 29.00                    |
| 3           | Anchovy                | Monday, 16-3-2015, 06.30 | 9.00                     |
| Processed   |                        |                        |                          |
| 4           | Salted striped snakehead | Saturday, 14-3-2015, 17.30 | 12.50                    |
| 5           | Salted anchovy         | Saturday, 14-3-2015, 17.30 | 282                      |
| 6           | Salted squid           | Sunday, 15-3-2015, 11.30 | 131                      |
| 7           | Salted yellowstripe scad | Sunday, 15-3-2015, 11.30 | 29.00                    |
| 8           | Acetes                 | Sunday, 15-3-2015, 11.30 | 209                      |
| 9           | Shrimp paste           | Sunday, 15-3-2015, 11.30 | \( \leq 10 \)           |
| 10          | Salted boiled fish     | Monday, 16-3-2015, 07.30 | 49.00                    |
| 11          | Boiled milkfish        | Saturday, 14-3-2015, 17.00 | 54.00                    |
| 12          | Boiled mackarel        | Monday, 16-3-2015, 07.30 | 38.00                    |
| 13          | Boiled mackarel        | Saturday, 14-3-2015, 17.00 | 17.00                    |
| **Modern markets** |                         |                        |                          |
| Fresh       |                        |                        |                          |
| 14          | Bangka Squid           | Friday, 13-3-2015, 19.00 | 127                      |
| 15          | Tilapia                | Friday, 13-3-2015, 19.00 | 22.50                    |
| 16          | Salmon fillet          | Friday, 13-3-2015, 19.00 | 103                      |
| 17          | Vannamei shrimp        | Friday, 13-3-2015, 19.00 | 41.50                    |
| 18          | Banjar milkfish        | Friday, 13-3-2015, 19.00 | 20.50                    |
| Processed   |                        |                        |                          |
| 19          | Unsalted anchovy       | Friday, 13-3-2015, 19.00 | 91.5                     |
| 20          | Shrimp paste           | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
| 21          | Crab Claw              | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
| 22          | Otak-otak (traditional food) | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
| 23          | Squid roll             | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
| 24          | Chikuwa                | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
| 25          | Salmon ball            | Friday, 13-3-2015, 19.00 | \( \leq 10 \)           |
Figure 1. Melting curve of the ompC gene of *Salmonella typhimurium* (ATCC 25241).

Figure 2. Melting curve of the real-time PCR product from twenty-five seafood products.

Early detection of *Salmonella* bacteria on food products is important to prevent hazards on human health. To date, over 2500 *Salmonella* serotypes have been identified. Of these, *Salmonella enterica* serovar typhimurium (*S. Typhimurium*) and *S. enterica* serovar Enteritidis (*S. Enteritidis*) are the most common caused of foodborne illness. Due to this high variability of *Salmonella* serovar, nucleic acid-based early detections is preferred. These techniques utilize a specific sequence of *Salmonella* DNA, resulting in an accurate detection. Furthermore, employing very advance real-time PCR method could improve the detection system by automating DNA extraction, amplification, and detection (Lee et al 2015).

In conclusion, real-time PCR was successfully developed to amplify the ompC gene of *Salmonella*. None of the seafood products tested was found to be contaminated by *Salmonella* bacteria.
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