A Real-Time PCR Detection of Genus *Salmonella* in Meat and Milk Samples

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Abstract

The aim of this study was follow the contamination of ready to eat milk and meat products with *Salmonella* spp. by using the Step One real-time PCR. Classical microbiological methods for detection of food-borne bacteria involve the use of pre-enrichment and/or specific enrichment, followed by the isolation of the bacteria in solid media and a final confirmation by biochemical and/or serological tests. We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and SensiFAST SYBR Hi-ROX Kit for the real-time PCR performance. In the investigated samples without incubation we could detect strain of *Salmonella* sp. in five out of twenty three samples (swabs). This Step One real-time PCR assay is extremely useful for any laboratory in possession of a real-time PCR. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future. Our results indicated that the Step One real-time PCR assay developed in this study could sensitively detect *Salmonella* spp. in ready to eat food.

Keywords: detection kit, food, Real-time PCR, *Salmonella* spp.

1. Introduction

Classical microbiological methods for detection of foodborne bacteria involve the use of pre-enrichment and/or specific enrichment, followed by the isolation of bacteria in solid media and a final confirmation by biochemical and/or serological tests [1]. In recent years, PCR-based methods have been reported as a rapid, specific and sensitive alternative, and have been increasingly used to identify several microbial species from food, [2] and clinical samples [3]. To speed up the analysis, PCR and more recently real-time PCR have been applied in various stages of the diagnostic procedure: confirmation of suspected colonies grown on agar plates, analysis of enrichment broths, and direct analysis of suspected foodstuffs. A large variety of target sequences and detection formats using commercial kits as well as non-patented methods have been used for this purpose [4-7].

The project focused on PCR detection methods for five major foodborne pathogens: *Campylobacter* spp., *Escherichia coli O157, Yersinia enterocolitica, Listeria monocytogenes,* and *Salmonella* spp. Direct detection of pathogens in food by PCR is still far from being a routine procedure. This is because of the low concentration of bacteria in food and the PCR-inhibitory effects of complex food matrices. Therefore, the project focused on the detection of these pathogens in enrichment broths. Conventional PCR requires post-PCR gel electrophoresis analysis to confirm the presence of the target in the sample. In contrast, the real-time PCR method is based on an increase in fluorescence, which indicates the presence of the target and is monitored during the PCR, thus requiring no post-PCR handling of the sample and reducing the risk of a false positive result due to contamination in the laboratory. In the present study we describe a real-time PCR approach using

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a TaqMan® probe for the detection and quantification of Salmonella spp., based on an internationally validated conventional PCR system targeting the invA gene. This PCR assay was suggested as a standard method for the detection of Salmonella spp. in the FOOD-PCR project [2].
The aim of this study was to follow the contamination of food of animal origin with *Salmonella* spp. by Step One real-time PCR.

2. Materials and methods

Food samples

A total of 23 samples of foods of animal origin were used in this study. The samples (5 pcs of Tourist salami, 4 pcs of Čingovská salami, 4 pcs of Inovecká salami and 10 pcs of raw milk) were analyzed for the presence of *Listeria monocytogenes*. Samples were obtained by taking swabs from the food of animal origin.

General Sample Preparation Protocol

Sample of 750 µL was loaded onto the spin column and microcentrifuged for 3 minutes at maximum speed. Supernatant was discarded and 50 µL of Lysis Buffer was added to the pellet. Samples were incubated for 10 minutes at 95 °C.

Measuring the concentration of DNA-UV-spectrophotometric quantification of DNA

The absorbance at a wavelength of 260 nm was measured and DNA concentration was calculated on base of the observation that double stranded DNA solution with a concentration of 5 $\mu g.mL^{-1}$ has a density about 0.1. To determine the contamination of the protein preparation the additional measuring absorbance at 280 nm was used. Preparation is considered as uncontaminated if proteins A260/A280 are between 1.8 and 2.0. Spectrophotometric measurements were done using UV 1101 photometer (Biotech, UK).
Thermal cycling conditions were as follows: 3 minutes of incubation at 95 °C, followed by 40 cycles of 5 sec. denaturation at 95°C and 15 sec. annealing and elongation at 60°C. Data were collected during each elongation step.

Reaction mix composition

Sensi Fast sybr Hi-ROX Mix	10 µL
Forward Primer	0.8 µL
Reverse Primer	0.8 µL
H_2O	9.4 µL
Template	4 µL

Primer name:

- Stn P1 5' TTG TGT CGC TAT CAC TGG CAA CC 3' a Stn M 13 5' ATT GTT AAC CCG CTC TCG TCC 3'.

PCR products were detected by monitoring the increase in fluorescence of the reporter dye at each PCR cycle. Applied Biosystems software plots the normalized reporter signal, ΔRn, (reporter signal minus background) against the number of amplification cycles and also determines the threshold cycle (Ct) value i.e. the PCR cycle number at which fluorescence increases above a defined threshold level.

3. Results and discussion

The most sensitive detection of *Salmonella* spp. was obtained using PrepSEQ™ Rapid Spin Sample Preparation Kit and SensiFAST SYBR Hi-ROX Kit. A detection kit compatible with StepOne™ Systems was less time-consuming than the other methods and relatively easy to use. Thus, the PCR-based detection of bacteria depends on the efficiency of DNA extraction procedure used to prepare the template DNA. In our samples without incubation we could detect strain of *Salmonella* spp. in 5 out of 23 samples (swabs), as it is shown in Figure 1. The (Ct) value of positive salmonella samples was on average 18.09, whereby the lowest value of positive salmonella samples was found at 15.86 and the highest value was at 23.99. Until now, many real-time PCR methods have been described evaluating their sensitivity and specificity [8-12]. However, only few papers describe the precision and accuracy of the methods evaluated. Even less papers are available presenting comparative studies of different real-time PCR methods for the detection of a specific organism [13], and none for detection of Salmonella spp. or E. coli O157:H7.
These results suggest that the detection limit of the assay is about one copy of the invA gene per PCR. Analysis of a large number of replicates and application of the Poisson statistics could be used in order to further characterize the detection limit [14].

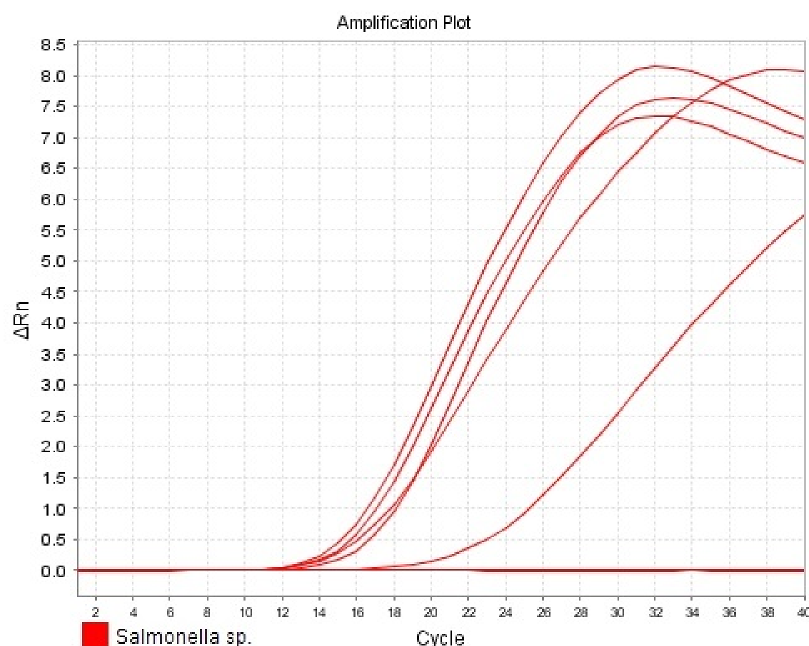

Figure 1. Contamination of food with *Salmonella* spp.

Still, using an internal amplification control with real-time PCR detection is important to identify false negative results and to control for presence of amplification inhibitors, especially for certified routine diagnostic laboratories [3, 13]. Previously, real-time PCR assays had been applied for *Salmonella* spp. and other food-borne pathogens [14]. In themultiplex real-time PCR, *Salmonella* spp., *S.* Typhimurium and *S.* Enteritidis produced mean CT values of 13.00 ± 0.94, 18.29 ± 2.23 and 16.39 ± 3.38, respectively for the specificity of the multiplex real-time PCR. When 0.54 ± 0.09 log10 CFU/mL of *S.* Typhimurium and 0.65 ± 0.07 log10 CFU/mL of *S.* Enteritidis were spiked into meats, the multiplex real-time PCR assay could detect bacteria in the spiked beef but not in the spiked pork, both from a preenrichment condition. The multiplex real-time PCR assay detected bacteria that underwent a post-enrichment condition. Pre-enriched meats had CT values of 35.32 or more, while post-enriched meats had CT values of 14.41 to 22.23. When 1.45 ± 0.21 log10 CFU/mL of *S.* Typhimurium and 1.65 ± 0.07 log10 CFU/mL of *S.* Enteritidis were spiked into meats, the multiplex real-time PCR assay detected bacteria in all conditions (beef, pork, pre-enriched, and post-enriched). However, pre-enriched meats had CT values from 30.94 to 36.94, while post-enriched meats had CT values ranging from 14.52 to 22.06 [15].

4. Conclusions

In conclusion, the present study reports a sensitive and specific real-time PCR assay using non-patented primers and a TaqMan® probe for the detection of *Salmonella* spp., based on a previously validated conventional PCR assay. The assay performed equally well as a commercially available kit and allowed sensitive detection of *Salmonella* spp. in chicken meat, salmon, and raw milk. Our results indicate that the StepOne realtime PCR assay developed in this study could sensitively detect *Salmonella* spp. in food of animal origin. This could prevent many people frombecoming infected with *Salmonella*.

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