International Measurement Confederation (IMEK0)
TC 23 "Metrology in Foods and Nutrition"
7th IMEKOFOODS International Conference. Worldwide food trade and consumption: quality and risk assessment
25-27 October 2023, Maisons-Alfort/Paris, France

Modification of real-time PCR method for detection of enterotoxin genes *sea-see* in *Staphylococcus aureus* isolates from food

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Staphylococcal food poisoning is one of the most common food-borne diseases worldwide. The causative agents are enterotoxins produced by enterotoxigenic strains of *Staphylococcus aureus* during its growth in favorable conditions in food. Since not all *S. aureus* strains can produce enterotoxins, detection of the enterotoxigenic ones is important for risk assessments and epidemiological investigations. Many molecular methods have been developed for the detection of staphylococcal enterotoxin genes. This work describes a modification of the original real-time PCR method for the detection of staphylococcal enterotoxin *sea-see* genes described by Nakayama et al. (2006).

The original real-time PCR method was based on 5' nuclease real-time simplex PCR with the use of patented fluorogenic probes. Modifications of the original PCR protocol included an adjustment to Thermo Fisher Scientific PikoReal 24 instrument, a smaller reaction volume (20 µL), different reaction components (Thermo Fisher Scientific Luminaris Master mix), and a smaller number of cycles (40). Method efficiency was determined based on the standard curves of target genes (*sea-see*). Real-time PCR was performed on ten-fold serial dilutions of each of the target gene DNA in triplicate. Standard curves were constructed based on threshold cycle (Ct) values versus log values of DNA concentrations. Slope value was used for the calculation of efficiency according to the equation. The intercept of the Y-axis determined the limit of detection in terms of Ct values. The reproducibility of each PCR reaction was calculated as standard deviation and inter-assay coefficient of variation.

Standard curves showed a linear relationship between Ct values and log values of DNA concentrations with very good data correlation (R^2 values between 0.995 and 0.999). The detection limits were at the following Ct values: 39.4 for *sea*, 39.5 for *seb*, 41.1 for *sec*, 37.9 for *sed*, and 39.3 for *see* gene. The efficiency of PCR reactions was in the optimal range; 97.0%,105.1%, 101.0%, 100.6%, and 99.1%, *sea* to *see* respectively. The inter-assay coefficient of variation was between 0.24% and 4.38%. The modified method proved to be very efficient, reproducible, and discriminatory between positive and negative results.

Keywords: real-time PCR, *Staphylococcus aureus*, staphylococcal enterotoxin genes, cheese

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Reference:

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