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Quantification of *Staphylococcus aureus* in white cheese by the improved DNA extraction strategy combined with TaqMan and LNA probe-based qPCR

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ABSTRACT

Four different bacterial DNA extraction strategies and two different qPCR probe chemistries were studied for detection of *Stapylococcus aureus* from white cheeses. Method employing trypsin treatment followed by a commercial kit application and TaqMan probe-based qPCR was the most sensitive one detecting higher counts than standards in naturally contaminated samples.

1. Introduction

Staphylococcus aureus infections and intoxications related to consumption of contaminated cheese have significance for product quality and public health (Temelli et al., 2006, Morandi et al., 2007). The European Food Safety Authority (EFSA) reported that cheeses with mixed and buffet meals were the two main ways involved in food poisoning outbreaks caused by staphylococcal toxins (Schelin et al., 2011). In addition, in Turkey, the most widely consumed cheese type is Turkish white cheese with an approximate production rate of 67% (Temelli et al., 2006). There have been several reports of *S. aureus* contamination in cheeses and other foods (Hein et al., 2005; Peles et al., 2007; Huong et al., 2010; Ertas et al., 2010; Alaboudi et al., 2012; Hummerjohann et al., 2014).

Cheese can be an important source of *S. aureus* contamination through raw milk, inadequate pasteurization process and improper conditions after pasteurization or production area (Miranda et al., 2009). For evaluating the safety of food production process, it is important to quantify the changes in microbial numbers (Schelin et al., 2011). Traditional microbiological methods have several drawbacks such as long detection time, high cost, and low sensitivity (Cremonesi et al., 2007). Quantitative PCR (qPCR) is increasingly used as a rapid, specific, and sensitive method for reliable detection and accurate gene quantification (Ginzinger, 2002). The advantages of qPCR are high sensitivity, high specificity, and lower risks of cross-contamination (Abdunaser et al., 2009). Several studies have been performed by qPCR to detect and quantify different bacterial cells in food products (D'Urso et al., 2009; Martínez-Blanch et al., 2009; Pennacchia et al., 2009). Several studies have been carried out on detection of *S. aureus* in food samples by qPCR method (Alarcón et al., 2006; Fusco et al., 2011; Hein et al., 2001; Poli et al., 2007). Cheese is a difficult matrix for direct *S. aureus* detection and quantification by qPCR method due to its high fat content (Ercolini et al., 2004). There have been few studies on the development of DNA extraction methods directly from food samples for detection of *S. aureus*. In one of these studies 33 bovine and caprine raw milk cheese samples were investigated and DNA extraction method was developed with a detection level of 100 CFU/g for *S. aureus* (Cremonesi et al., 2007).

Different PCR technologies have been developed for better detection purposes mainly based on novel primer and probe chemistries. Two important chemistries that are used in qPCR systems are TaqMan and LNA probes that specifically bind to the target sequence (Gachon et al., 2004). Locked nucleic acid (LNA) is the analogue of a nucleic acid described by the Wengel and Imanishi laboratories (Letertre et al., 2003). The C3′-endo type sugars are conformationally locked by a methylene bridge between 2′ oxygen and 4′ carbon of the ribose ring (Jepsen

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et al., 2004). The flexibility of the ribofuranose ring is reduced by this methylene bridge causing the formation of a rigid bicyclic monomer. This also increases the local organization of the phosphate backbone and result in strong hybridization between two DNA strands (Letertre et al., 2003; Reynisson et al., 2006). To the best of our knowledge, the studies on direct quantitative detection of *S. aureus* in Turkish cheese samples are very limited. In addition, optimization of a method for one food system may not be applicable to other food samples (Federico et al., 2005). The purpose of this study is to improve the detection sensitivity of *S. aureus* by experiencing different bacterial DNA extraction methods directly isolated from cheese samples. In addition, the comparison of TaqMan and LNA probes in the qPCR assays for the sensitive detection and quantification of *S. aureus* bacterial cells by targeting species specific *nuc* gene was proposed in artificially and naturally contaminated Turkish white cheese samples.

2. Materials and method

2.1. Bacterial strain and DNA isolation

S. aureus RSKK 1009 strain was used to prepare quantification standard and for artificial inoculation of cheese samples. The reference strain was grown in 6 mL of Tryptic soy broth (Merck, Darmstadt, Germany) and incubated at 37 °C for 18 h. The turbidity of bacterial suspension in Tryptic soy broth was adjusted to 0.5 McFarland (10^7 CFU/mL). Tenfold serial dilutions were prepared from this suspension. Colonies were enumerated by plating onto Baird Parker Agar (BD-Difco™, France) supplemented with egg yolk tellurite emulsion (BBL®, USA). DNA extraction from the pure culture was performed according to the method described previously (Sudagidan et al., 2008).

2.2. Artificially and naturally contaminated white cheese samples

The detection ranges of TaqMan and LNA probes in artificially contaminated cheese samples were determined by inoculating tenfold serial dilutions of pure culture to white cheese samples purchased from a local supermarket in İzmir and they were originally free of *S. aureus*. Four different bacterial DNA extraction procedures were carried out directly from these samples.

For quantification of *S. aureus* in naturally contaminated cheese samples, cheese samples (n = 7) were collected from different bazaars in İzmir district (Turkey) and analyzed immediately using traditional culture method and incubated at 37 °C for 24–48 h. The traditional plate count method included homogenization of 25 g sample in 225 mL buffered peptone water and plating 0.1 mL aliquots in Baird Parker agar supplemented with egg yolk tellurite emulsion and incubation at 37 °C for 24–48 h.

2.3. Bacterial DNA isolation from cheese samples

For quantitative detection of *S. aureus*, the bacterial DNA was extracted from artificially contaminated cheese with four different methods (Fig. 1). The main steps were followed according to the previously given procedures, but significant modifications were applied to improve the sensitivity of detection. The C_t values obtained using these isolation methods were analyzed by using Fisher's test and analysis of variance (ANOVA) using MINITAB® release 14 (Minitab Inc., State College, USA).

2.3.1. Bacterial DNA extraction using the food DNA extraction kit (Method 1)

A commercial kit based on silica-gel membrane technology for rapid and efficient purification of DNA without organic extraction and ethanol precipitation (Intron Biotechnology, Inc., Korea) was applied according to the manufacturers' instructions. The steps in this procedure included lysis with lysis buffer, proteinase K and RNase A, precipitation, binding, and washing steps with buffer solutions. After selective binding of DNA to the column, the final eluate was recovered with elution buffer.

2.3.2. Bacterial DNA extraction using beads (Method 2)

The bacterial DNA extraction from artificially contaminated cheese samples was carried out using the procedure assessed by Bonaiti et al. (2006) with slight modifications. Briefly, 2 g of cheese sample was

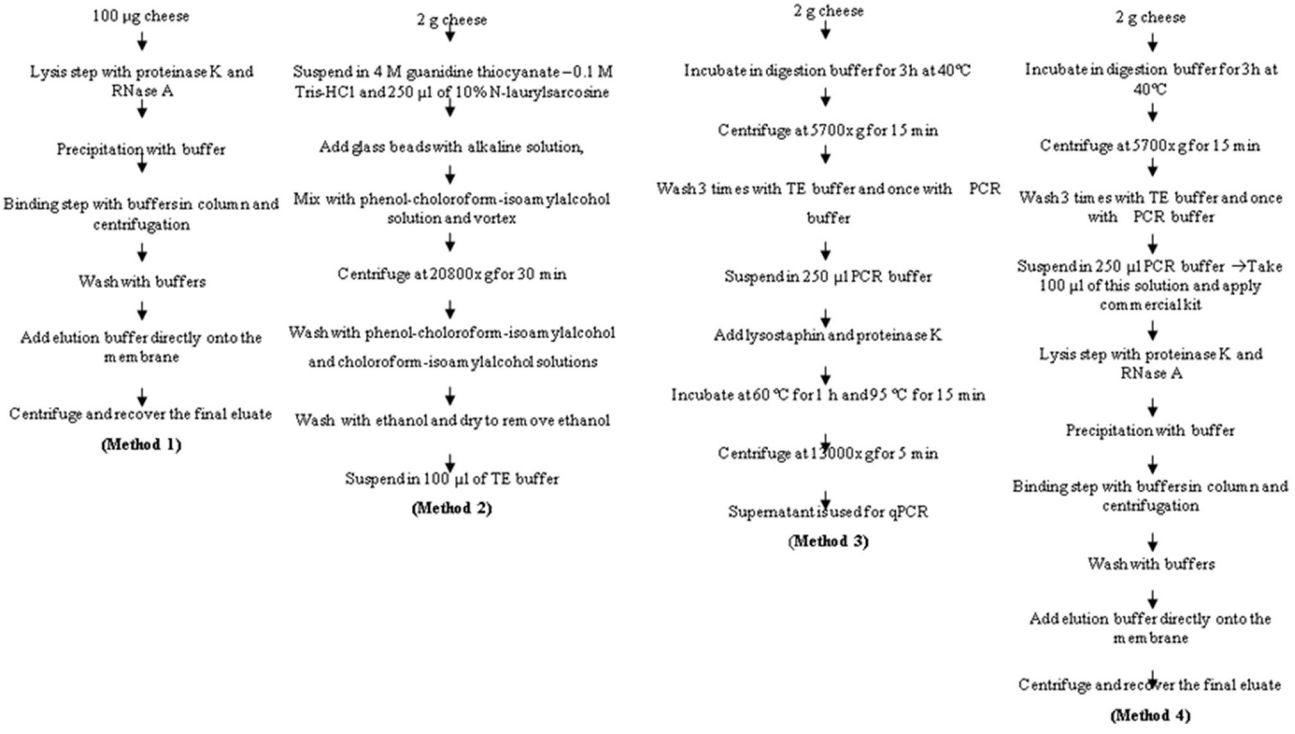

Fig. 1. Methods used for DNA extraction from cheese samples.