J. Dairy Sci. 101:1–8
https://doi.org/10.3168/jds.2017-14087

Quantitative polymerase chain reaction coupled with sodium dodecyl sulfate and propidium monoazide for detection of viable *Staphylococcus aureus* in milk

Lei Dong,*†‡[1] Huimin Liu,*†§[1] Lu Meng,*†[1] Mengru Xing,*†‡ Jiaqi Wang,*† Cheng Wang,§ He Chen,§ and Nan Zheng*†[2]

*Ministry of Agriculture Laboratory of Quality and Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China
†Ministry of Agriculture—Milk and Dairy Product Inspection Center (Beijing), Beijing 100193, P. R. China
‡College of Food Science and Engineer, Qingdao Agricultural University, Qingdao 266109, P. R. China
§Institute of Quality Standard and Testing Technology, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, P. R. China

ABSTRACT

Conventional quantitative PCR (qPCR) are unable to differentiate DNA of viable *Staphylococcus aureus* cells from dead ones. The aim of this study was to use sodium dodecyl sulfate (SDS) and propidium monoazide (PMA) coupled with lysostaphin to detect viable *Staph. aureus*. The cell suspensions were treated with SDS and PMA before DNA extraction. The SDS is an anionic surfactant, which can increase the permeability of dead cells to PMA without compromising the viability of live cells. The lysostaphin was applied to improve the effectiveness of DNA extraction. The reliability and specificity of this method were further determined by the detection of *Staph. aureus* in spiked milk. The results showed that there were significant differences between the SDS-PMA-qPCR and qPCR when a final concentration of 200 μg/mL of lysostaphin was added in DNA extraction. The viable *Staph. aureus* could be effectively detected when SDS and PMA concentrations were 100 μg/mL and 40 μ*M*, respectively. Compared with conventional qPCR, the SDS-PMA-qPCR assay coupled with lysostaphin was more specific and sensitive. Therefore, this method could accurately detect the number of viable *Staph. aureus* cells.

Key words: propidium monoazide, sodium dodecyl sulfate, *Staphylococcus aureus*, quantitative polymerase chain reaction, internal amplification control

INTRODUCTION

Staphylococcus aureus is a spherical, gram-positive, pathogenic bacterium that can produce enterotoxins. *Staphylococcus aureus* is a major cause of bovine mastitis in dairy herds, and it causes cellulitis and osteomyelitis (Loozen et al., 2011; Zhang et al., 2015; Cortimiglia et al., 2016). The presence of *Staph. aureus* in raw milk is also a potential source of the pathogen in the dairy food chain, with consequent risk of food contamination (Guardabassi et al., 2013). High prevalence of *Staph. aureus* has been reported in some countries, such as the United States and Italy (Heidinger et al., 2009; Cortimiglia et al., 2016).

Therefore, accurate and rapid methods for detecting *Staph. aureus* are in urgent demand. Culture-based methods for detecting *Staph. aureus* are inexpensive and reliable but laborious and time consuming (Velusamy et al., 2010). Methods based on PCR technology could overcome these drawbacks of culture-based methods because of its specificity and sensitivity. However, it is not possible to distinguish viable cells from dead ones by using quantitative PCR (**qPCR**), which may lead to overestimates of *Staph. aureus* numbers (Wang and Levin, 2006).

According to permeability differences between viable and dead cells, nucleic acid dyes, such as propidium monoazide (**PMA**), can selectively penetrate membrane-damaged cells, bind to DNA by light activation, and sequentially inhibit amplification of the bound DNA in qPCR reactions. Therefore, only DNA from viable bacteria with intact membranes can be detected in PMA-qPCR systems (Nocker et al., 2006; Gensberger et al., 2014; Xiao et al., 2015; Truchado et al., 2016). However, some inhibitors from the sample matrix may influence the effectiveness of PMA, leading to overestimation of viable cells, which limits the application of the methods (Seinige et al., 2014a; Truchado et al., 2016).

Received November 3, 2017.
Accepted January 4, 2018.
[1]Lei Dong, Huimin Liu, and Lu Meng contributed equally.
[2]Corresponding author: zhengnan_1980@126.com

Sodium dodecyl sulfate can increase the permeability of dead cells to PMA without compromising the viability of live cells (Takahashi et al., 2017). Therefore, SDS could be used to treat *Staph. aureus* along with PMA. Lysostaphin is an antibacterial enzyme that specifically cleaves the peptidoglycan cross-linking pentaglycine bridges of *Staph. aureus*. It was used to hydrolyze the cell wall and lyse the bacteria (Francius et al., 2008).

The aim of this study was to improve and evaluate a novel qPCR assay coupled with SDS and PMA to detect and quantify viable *Staph. aureus* cells in milk. Moreover, optimization of SDS and PMA was designed to improve the detection effect of SDS-PMA-qPCR on viable *Staph. aureus* cells.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

In this study, *Staph. aureus* (ATCC6538) and *Bacillus cereus* (ATCC11778) were cultured in Luria-Bertani medium (Beijing Land Bridge Technology Ltd., Beijing, China) in a rotary shaker at 37°C for 24 h until the stationary phase was achieved. Aliquots (10 mL) of the suspension were first transferred to a 50-mL centrifuge tube (Corning Inc., Corning, NY) and centrifuged at 15,000 × *g* for 3 min at 4°C to harvest the cells. Then, the pellet was resuspended in sodium chloride solution (0.85%, Beijing Land Bridge Technology Ltd.). To determine the number of viable cells, 6 serial dilutions were prepared and 100-μL aliquots from the serial dilutions were spread onto Luria-Bertani agar. Bacterial counts were determined by counting colonies after the plates were incubated at 37°C for 24 h.

Genomic DNA Extraction

The bacteria pellet was first resuspended in TE buffer (10 m*M* Tris-HCl and 1 m*M* EDTA, pH 8.0), and then lysostaphin stock solution was added to a final concentration of 200 μg/mL. The mixture was incubated at 37°C for 1 h. Genomic DNA was extracted with the cetyl trimethyl ammonium bromide method. Rehydrated DNA was examined with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) to determine concentration and quality. All DNA samples were stored at –20°C until use.

Inclusivity and Exclusivity of Primer Tests

To examine inclusivity and exclusivity, tests were performed using a panel of 18 strains, including 10 *Staph. aureus* standard strains and other known foodborne pathogens (Table 1). The DNA templates were extracted using the cetyl trimethyl ammonium bromide method. The 25-μL reaction volume contained 12.5 μL of qPCR Master Mix (Sigma-Aldrich, St. Louis, MO), 2 μL of sample templates, 1 μL of each of the primers, and 8.5 μL of distilled H_2O. The primers were 5′-CACCTGAAACAAAGCATCCTAAA-3′ and 5′-CGCTAAGCCACGTCCATATT-3′, and the probe was 5′-TGGTCCTGAAGCAAGTGCATTTACGA-3′ (Li et al., 2015). The sequences of internal amplification control (**IAC**) used were 5′-CGCAAGGCTGAAACTCAAAG-3′ and 5′-GAGGATGTCAAGACCTGGTAAG, and the probe was 5′-ACAAGCGGTGGAGCATGTGGTTTA (Li et al., 2015). The cycling protocol included an initial 10-min denaturation step at 95°C followed by 40 cycles of repeated denaturation at 95°C for 15 s and annealing and extension at 62°C for 1 min. Fluorescent data were acquired during the annealing and extension phase. A negative control with water was included in each qPCR reaction. After amplification, PCR products were subjected to 2.5% agarose gel electrophoresis and visualized with a UV transilluminator (Bio-Rad, Hercules, CA) after staining with nucleic acid dye (Qiagen, Hilden, Germany).

Optimization of SDS Treatment on Bacteria

Treatment conditions were as described by Takahashi et al. (2017) with slight modification. The SDS stock solution (20% wt/vol) was prepared by dissolving SDS in 0.1% (wt/vol) peptone water and then sterilized. The bacteria suspensions were centrifuged at 5,000 × *g* for 10 min at 4°C. The pellets were then resuspended in 0.1% (wt/vol) peptone water with serially diluted SDS. The viability of bacteria in the SDS solutions with final concentrations of 0, 25, 50, 100, 250, 500, and 1,000 μg/mL was examined on appropriate plates. The loss of viability of cells was tested by plating 100 μL of cell suspension on agar plates at 37°C for 24 h. The concentration of SDS was optimized according to the results of plate counts for inhibition of amplification of dead *Staph. aureus* cells.

Preparation of Dead Cells

To prepare the dead cells, the bacteria cultures in the exponential phase were centrifuged at 5,000 × *g* for 10 min at 4°C, and the pellets were washed and suspended in 0.1% (wt/vol) peptone water. Bacterial suspensions were adjusted to cell concentration between 10^7 and 10^8 cfu/mL. The suspension was divided into 2 aliquots—one used to prepare dead samples at 90°C for 20 min (plate count was used to confirm that no bacteria had