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Rapid detection of *Staphylococcus aureus* in dairy and meat foods by combination of capture with silica-coated magnetic nanoparticles and thermophilic helicase-dependent isothermal amplification

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ABSTRACT

Staphylococcus aureus is one of the main pathogens in dairy and meat products; therefore, developing a highly sensitive and rapid method for its detection is necessary. In this study, a quantitative detection method for *Staph. aureus* was developed using silica-coated magnetic nanoparticles and thermophilic helicase-dependent isothermal amplification. First, genomic DNA was extracted from lysed bacteria using silica-coated magnetic nanoparticles and amplified using thermophilic helicase-dependent isothermal amplification. After adding the nucleic-acid dye SYBR Green I to the amplicons, the fluorescence intensity was observed using a UV lamp or recorded using a fluorescence spectrophotometer. This detection system had a detection limit of 5×10^0 cfu/mL in pure culture and milk-powder samples and 5×10^1 cfu/mL in pork samples using a UV light in less than 2 h. In addition, a good linear relationship was obtained between fluorescence intensity and bacterial concentrations ranging from 10^2 to 10^4 cfu/mL under optimal conditions. Furthermore, the results from contaminated milk powder and pork samples suggested that the detection system could be used for the quantitative analysis of *Staph. aureus* and applied potentially to the food industry for the detection of this pathogen.
Key words: *Staphylococcus aureus*, silica-coated magnetic nanoparticles, thermophilic helicase-dependent isothermal amplification, SYBR Green I

INTRODUCTION

Food-borne diseases caused by pathogens have attracted worldwide attention because almost a quarter of the population is currently at risk of suffering from food-borne illnesses (Omiccioli et al., 2009). *Staphylococcus aureus* is the second-most-prevalent food-borne pathogen that exists widely in the environment, particularly in food (Miao et al., 2010). A previous study reported that *Staph. aureus* accounted for 2.57% of the 9,388,075 food-borne illnesses per year in the United States (Scallan et al., 2011). In addition, reports suggest that about 30 to 50% of the population carries *Staph. aureus* (Lowy, 1998). Because of its prevalence and the associated hazard, a rapid and accurate method to detect *Staph. aureus* is crucial to prevent severe health problems.

The conventional standardized methods used to detect and identify *Staph. aureus* were based on cell culture and biochemical tests, including enrichment cultivation, selective cultivation, biochemical identification, and serological reactions. However, the practical applications of conventional methods are limited because they are laborious and time consuming (Gilbert, 2002). Recently, molecular diagnostics involving nucleic-acid amplification have become the most commonly used methods for bacterial detection (Lazcka et al., 2007). Polymerase chain reaction, a nucleic acid–based method, is used widely to detect *Staph. aureus* (Kim et al., 2001; Vancraeynest et al., 2007; Yang et al., 2007) because of its high sensitivity and specificity. Nevertheless, PCR normally requires precise temperature control during thermal cycling and a time-consuming temperature-ramping process (Wang et al., 2011). Furthermore, extensive sample preparation, specialized instruments, and technical expertise are needed.

Consequently, isothermal amplification techniques have attracted increasing attention in recent years and have been used successfully as an alternative tool for the rapid detection of pathogens (Gill and Ghaemi, 2008; Kim and Easley, 2011). Examples of such techniques include strand displacement amplification (Hellyer and Nadeau, 2004), rolling circle amplification (Haible et al., 2006), loop-mediated isothermal amplification

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(Li et al., 2013; Yang et al., 2013), and nucleic acid sequence-based amplification (Mollasalehi and Yazdanparast, 2013). Compared with PCR-based methods, isothermal nucleic-acid amplification has the potential to achieve low-cost molecular diagnosis because it is performed at a constant temperature, thereby eliminating the requirement for a thermocycler. However, most isothermal nucleic acid–amplification techniques still have limitations such as complicated reaction schemes and challenges amplifying DNA targets of sufficient length to be useful for various applications and diagnosis (Wang et al., 2011). Therefore, thermophilic helicase-dependent isothermal amplification (**tHDA**), a relatively novel isothermal amplification technology developed by BioHelix (Beverly, MA; Vincent et al., 2004) has been introduced. In the past 10 yr, tHDA has been used successfully to detect several pathogens, including *Escherichia coli* (Mahalanabis et al., 2010), *Helicobacter pylori* (Gill et al., 2008), *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* (Doseeva et al., 2011). However, tHDA-based methods have not yet been used to monitor target pathogens quantitatively.

In this study, we used tHDA to detect *Staph. aureus* quantitatively using nucleic-acid dye SYBR Green I (Invitrogen, Shanghai, China) as the signal reporter based on its high specificity for double-stranded DNA (**dsDNA**) and bright fluorescence upon intercalation into dsDNA. In addition, silica-coated magnetic nanoparticles (**Si-MNP**) were used to extract genomic DNA from food samples, which successfully avoided sample preincubation, and eliminated the influence of the food matrix. This platform could detect *Staph. aureus* even at low levels and exhibited specificity for this pathogen.

MATERIALS AND METHODS

Materials and Reagents

Silica-coated magnetic nanoparticles were provided by Wuxi Zodoboer Biotech. Co. Ltd. (Wuxi, China). The nucleic-acid dye SYBR Green I was obtained from Invitrogen. The DNA marker was purchased from Dalian TaKaRa Biotechnology Company Ltd. (Dalian, China). All culture media were purchased from Beijing LuQiao Company (Beijing, China). All other reagents were of analytical grade and were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO).

Strains and Cultivation

The strains used in this study are listed in Table 1. All strains were provided by the State Key Laboratory of Food Science and Technology, Nanchang University (Nanchang, China), and stored in 20% glycerol at −80°C. The *Staph. aureus* strains were cultured in Luria-Bertani broth at 37°C, and the remaining bacteria were cultured according to the growth requirements of the individual organisms. Bacterial cells were harvested and washed twice with phosphate buffer (PBS, 0.01 *M*, pH 7.4). The concentration of *Staph. aureus* was determined using colony counting.

Preparation of Staph. aureus–Spiked Samples

The whole milk powder and pork samples, which were free of *Staph. aureus*, were purchased from a local supermarket. One-gram aliquots of *Staph. aureus*–negative whole milk powder were reconstituted in 8 mL of sterile distilled water, and then 1-mL aliquots of serial 10-fold dilutions of *Staph. aureus* were added to the reconstituted whole milk powder to obtain concentrations ranging from 5×10^0 to 5×10^7 cfu/mL. Moreover, to evaluate the sensitivity of the method in the presence of non-*Staph. aureus* bacteria, 10^8 cfu/mL of each of *Escherichia coli*, *Listeria monocytogenes*, and *Lactobacillus plantarum* was also mixed into the reconstituted whole milk powder containing 5×10^0 to 5×10^7 cfu/mL *Staph. aureus*.

For pork samples, serially diluted *Staph. aureus* and 10^8 cfu/mL *E. coli*, *L. monocytogenes*, and *L. plantarum* were mixed with a minced sample of meat to a final meat concentration of 10% (wt/vol). Then minced pork samples containing different concentrations of *Staph. aureus* (ranging from 5×10^0 to 5×10^7 cfu/mL) were also obtained and used for DNA extraction. The concentration of *Staph. aureus* was determined using colony counting.

Genomic DNA Extraction Using Si-MNP

All DNA extractions were performed as previously described previously (Chen et al., 2014a) with some modifications. Briefly, 1-mL aliquots of *Staph. aureus* sample were boiled at 100°C for 10 min to release the genomic DNA completely. Subsequently, 10 μL of Si-MNP solution (10 mg/mL) and 500 μL of hydrosaline solution (3 *M* NaCl, 2 *M* KCl) were added into the tubes. The tubes were then incubated for 20 min at 24°C with gentle shaking (130 rpm), and the Si-MNP–DNA complexes were isolated magnetically from the medium by placing the tubes in the separator racks on a permanent magnet (0.4 T) for 3 min. After washing twice with 80% ethanol, 20 μL of sterile deionized water was added to dissolve the DNA adsorbed on the surface of Si-MNP. Finally, the Si-MNP in the suspensions were collected again using the magnetic separator, and the supernatants containing the desorbed DNA were