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

Xingxing Chen,^{*1} Xiaoli Wu,^{†1} Min Gan,^{*} Feng Xu,^{*} Lihua He,^{*} Dong Yang,^{*} Hengyi Xu,^{*} Nagendra P. Shah,^{‡2} and Hua Wei^{*2}

^{*}State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, 330047, China

[†]School of Basic Medical Science, Jiangxi University of Chinese Traditional Medicine, Nanchang, Jiangxi, 330000, China

[‡]Food and Nutritional Science, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong

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

Received September 6, 2014.

Accepted November 11, 2014.

¹These authors contributed equally.

²Corresponding authors: weihua114@live.cn and npshah@hku.hk

(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 Zodober 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 Univer-

sity (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

obtained and stored at -20°C before amplification. All experiments were repeated in triplicate.

***Staph. aureus* Primers and the Capture Efficiency of Si-MNP**

The primers used to amplify *Staph. aureus* were as follows: sau-F, 5'-CAAGATGTGCACAGTTACTTACACATATG-3', and sau-R, 5'-CACTTATAGATG-GATCCGCGCT-3'. They were designed based on the conserved 16S rRNA gene sequences identified in GenBank, and their specificity for *Staph. aureus* was verified previously (Chen et al., 2014b).

To evaluate the capture efficiency of the Si-MNP, real-time PCR was performed on a 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA) using a SYBR Green PCR kit (TaKaRa) and performed in a 20- μL reaction mixture containing 10 μL of $2 \times$ SYBR *Premix Ex Taq* II buffer, 1 μL (0.1 nM) of primer sau-F/R primers, and 2.5 μL of DNA templates, and the volume was adjusted by using sterile deionized water. Cycling parameters were as follows: an initial denaturation at 95°C for 10 min, 42 cycles of 95°C (5 s), 62°C (60 s), and 72°C (20 s), and a final elongation at 72°C for 10 min. Each sample was run in triplicate, including a blank with no target DNA. The assays were run in triplicate.

tHDA

The tHDA reactions were performed using a commercially available kit (IsoAmp II Universal tHDA Kit, BioHelix). The one-step 50- μL tHDA reaction contained 5 μL of $10 \times$ annealing buffer; 2 μL of MgSO_4 (100 mM), 4 μL of NaCl (500 mM), 3.5 μL of IsoAmp:emoji: dNPT solution, 0.75 μL of each forward and reverse primer (5 μM), 3.5 μL of IsoAmp:emoji: enzyme mix, and 1 μL of test template. The volume was adjusted using sterile deionized water. All reaction mixtures were covered with 50 μL of mineral oil to avoid evaporation (Picard et al., 2005), and the tubes were placed on ice for 1 min. The reaction was incubated at 65°C for 90 min.

Assay Procedure

The assay used for the fluorescent detection of the target pathogen is illustrated in Figure 1. One milliliter of contaminated *Staph. aureus* samples was treated with 10 μL of Si-MNP solution (10 mg/mL) to purify and enrich the target genomic DNA, and 20 μL of the target *Staph. aureus* gene was then amplified using tHDA according to the manufacturer's protocol. Subsequently, 6 μL of SYBR Green I (19.6 mM) was added to the

Table 1. The bacterial strains used in experiment and results of specificity of the protocol

No.	Species and strain	Detected by tHDA ¹
1	<i>Staphylococcus aureus</i> PSAV0001	+
2	<i>Staph. aureus</i> PSAV0002	+
3	<i>Staph. aureus</i> PSAV0007	+
4	<i>Staph. aureus</i> PSAV0012	+
5	<i>Staph. aureus</i> PSAV0021	+
6	<i>Staph. aureus</i> PSAV0024	+
7	<i>Staph. aureus</i> PSAV0027	+
8	<i>Staph. aureus</i> PSAV0035	+
9	<i>Staph. aureus</i> PSAV0040	+
10	<i>Staph. aureus</i> PSAV0202	+
11	<i>Staph. aureus</i> PSAV0205	+
12	<i>Staph. aureus</i> PSAV0207	+
13	<i>Staph. aureus</i> PSAV0209	+
14	<i>Staph. aureus</i> PSAV0210	+
15	<i>Staph. aureus</i> PSAV0213	+
16	<i>Staph. aureus</i> PSAV0214	+
17	<i>Staph. aureus</i> PSAV0216	+
18	<i>Staph. aureus</i> PSAV0218	+
19	<i>Staph. aureus</i> PSAV0219	+
20	<i>Staph. aureus</i> PSAV0220	+
21	<i>Staph. aureus</i> CMCC26001	+
22	<i>Staph. aureus</i> CMCC26002	+
23	<i>Staph. aureus</i> CMCC26003	+
24	<i>Salmonella</i> Typhimurium ATCC 13311	—
25	<i>Pseudomonas aeruginosa</i> CMCC10104	—
26	<i>Salmonella</i> Enteritidis CMCC 50041	—
27	<i>Shigella flexneri</i> ATCC29903	—
28	<i>Escherichia coli</i> O157:H7 PELI1264	—
29	<i>E. coli</i> O157:H7 PELI0480	—
30	<i>Cronobacter sakazakii</i> ATCC 29544	—
31	<i>Cronobacter</i> spp. CICC 21544	—
32	<i>Shigella sonnei</i> ATCC 29930	—
33	<i>Listeria monocytogenes</i> CMCC54001	—
34	<i>L. monocytogenes</i> PLMO1448	—
35	<i>L. monocytogenes</i> PLMO1344	—
36	<i>Lactobacillus plantarum</i> ATCC 8014	—
37	<i>Lactobacillus acidophilus</i> ATCC 4356	—
38	<i>Enterococcus faecalis</i> ATCC 19433	—
39	<i>Campylobacter jejuni</i> NC002163	—
40	<i>Vibrio parahaemolyticus</i> 1616	—
41	<i>Bifidobacterium breve</i> WBBR04	—
42	<i>Bifidobacterium animalis</i> WBBR05	—
43	<i>Bifidobacterium bifidum</i> WBBI01	—
44	<i>Bifidobacterium longum</i> WBLO01	—
45	<i>Bifidobacterium infantis</i> WBAN07	—
46	<i>Bifidobacterium adolescentis</i> WBAD08	—
47	<i>Bifidobacterium lactis</i> WLABO9	—
48	<i>Lactobacillus rhamnosus</i> GG ATCC 7469	—
49	<i>Lactobacillus bulgaricus</i> F1	—
50	<i>Candida albicans</i> Z1	—

¹tHDA = thermophilic helicase-dependent isothermal amplification; + indicates positive signal; — indicates negative signal.

amplification product, incubated for target nucleic-acid detection, and then detected qualitatively under a UV light.

For quantitative detection, specific parameters, such as the concentration of SYBR Green I (5, 6, 7, 8, 9, and 10 μL), incubation time (1, 3, 5, 7, and 9 min), and amplification time (50, 60, 70, 80, and 90 min) were optimized. The fluorescence intensities of 3 replicates

Figure 1. Overall process of Si-MNP-tHDA-SYBR Green I assay for detection of *Staphylococcus aureus*. Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. SYBR Green I (Invitrogen, Shanghai, China). Color version available online.

for each target bacterial concentration were collected using an F-380 spectrophotometer (Shimadzu, Tokyo, Japan) equipped with a 150-W xenon lamp (Ushio Inc., Tokyo, Japan) with excitation at 495 nm and emission at 520 nm. A calibration curve was constructed by plotting the fluorescence value against the *Staph. aureus* concentrations obtained under the optimized conditions. The amount of *Staph. aureus* in the milk powder and pork samples was calculated using the formula $R = C/A$, where R is the recovery of *Staph. aureus*, C is the concentration of *Staph. aureus* determined using the calibration curve, and A is the adsorption efficiency of the Si-MNP. The experiments were carried out in triplicate.

Statistical Analysis

Results were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL) by means of independent one-way ANOVA tests in each sampling point. The differences among the 3 groups were assessed by means of the least significant difference multiple comparison test ($P < 0.05$).

RESULTS AND DISCUSSION

Capture Efficiency of the Si-MNP for *Staph. aureus* Genomic DNA

To evaluate the ability of Si-MNP to capture target genomic DNA in spiked samples, different concentrations of spiked samples containing *Staph. aureus* (rang-

Figure 2. Real-time PCR evaluation of the adsorption efficiency of the silica-coated magnetic nanoparticles in milk powder and pork samples (cell counts of *Staphylococcus aureus* ranging from 5×10^2 to 5×10^5 cfu/mL). Error bars indicated the deviation range in triplicate test results ($P > 0.05$).

ing from 10^2 to 10^5 cfu/mL) were used to capture *Staph. aureus* genomic DNA, which was then analyzed using real-time PCR. As shown in Figure 2, more than 50% of the *Staph. aureus* DNA fragments were recovered by Si-MNP in the spiked milk powder and pork samples. The recovery rate of DNA decreased with increasing *Staph. aureus* concentrations. As expected, the recovery rate of DNA in milk-powder samples was slightly higher than was that in pork samples.

Generally, the enrichment of DNA by Si-MNP occurred via a nonspecific absorption process, so the presence of nontarget DNA in the sample partially affected the efficiency of the enrichment procedure. Therefore, the DNA present in pork tissue might competitively occupy the binding sites of Si-MNP, thereby lowering the lower capability for DNA enrichment. Therefore, we verified that the recovery rate of DNA in milk-powder samples was slightly higher than was that from pork samples in the current study. Compared with the traditional DNA extraction methods such as phenol-chloroform extraction, the Si-MNP method avoids the time-consuming and laborious process of preculturing of the target pathogen. It also eliminates interference from components including ions, proteins, and fatty matter in samples.

Specificity of the Protocol

To assess the specificity of the protocol, 23 *Staph. aureus* and 27 non-*Staph. aureus* strains commonly used in food fermentation and frequent bacterial contaminants in food were amplified using tHDA and observed under UV light. As shown in Table 1, all 23 *Staph. aureus*

Figure 3. Detection limit of the Si-MNP-tHDA-SYBR Green I assay using serially diluted *Staphylococcus aureus* in pure culture. Lanes 1 to 8 = from 5×10^0 to 5×10^7 cfu/mL; N = negative control. All samples were repeated in triplicate. Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. SYBR Green I (Invitrogen, Shanghai, China). Color version available online.

strains gave positive signals, whereas the 27 non-*Staph. aureus* strains generated no signal. Therefore, the protocol appeared to be highly specific for the detection of *Staph. aureus*.

Detecting *Staph. aureus* in Pure Cultures

The performance of the novel protocol in pure cultures was evaluated using different concentrations of *Staph. aureus* (ranging from 10^0 to 10^7 cfu/mL). As shown in Figure 3, 10^0 cfu/mL of *Staph. aureus* could be visualized under a UV lamp. The tHDA amplification products were also analyzed using gel electrophoresis; however, the maximum detection limit was only 10^4 cfu/mL (Figure 4).

The results of gel electrophoresis demonstrated that the target DNA was amplified successfully and that the current SYBR Green I-based DNA detection method

Figure 4. Detection limit of Si-MNP-tHDA-agarose gel electrophoresis using serially diluted *Staphylococcus aureus* in pure culture. Lane M = DL2000 marker; lanes 1 to 5 = from 5×10^0 to 5×10^4 cfu/mL; N = negative control. Size of the target gene was about 263 bp. Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. The arrow indicates the detection limit by gel electrophoresis; the band in lane 3 is weak but real.

was more sensitive than that of gel electrophoresis. Loop-mediated isothermal amplification, a most common isothermal amplification technique, was also used to detect *Staph. aureus*. It had a detection limit of 8 to 10 cfu/mL (Niessen et al., 2013), which was similar to that of the tHDA protocol. However, loop-mediated isothermal amplification assays normally require 6 primers, which increase the complexity of the experiment. Moreover, the novel assay did not require any special processing or electrophoresis to identify the positive reaction.

Application of the Method in Spiked Milk Powder and Pork Samples

To evaluate the feasibility of the assay for detecting *Staph. aureus* in food samples, milk powder and pork samples were spiked because of the lack of pathogens in these samples. As shown in Figure 5A, the detection limit in milk-powder samples spiked with mixture of 10^8 cfu/mL of each *E. coli*, *L. monocytogenes*, and *L. plantarum* remained 10^0 cfu/mL of *Staph. aureus*. Similarly, the detection limit was 10^1 cfu/mL in pork samples (Figure 5B). These results indicate that a good specificity and sensitivity for the detection of *Staph. aureus* in food samples was achieved, even in the presence of interference from high concentrations of non-*Staph. aureus* bacteria.

The Si-MNP-tHDA-SYBR Green I assay used to detect *Staph. aureus* was more sensitive in milk powder compared with pork samples. This is consistent with

Figure 5. Detection limit of the Si-MNP-tHDA-SYBR Green I assay using serially diluted *Staphylococcus aureus* mixed with 10^8 cfu/mL of nontarget bacteria cells (*Escherichia coli*, *Listeria monocytogenes*, and *Lactobacillus plantarum*) in powder milk samples (A) and pork samples (B). Lanes 1 to 6 = from 5×10^0 to 5×10^6 cfu/mL; N = negative control. Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. SYBR Green I (Invitrogen, Shanghai, China). Color version available online.

Figure 6. Influence of concentrations of SYBR Green I (Invitrogen, Shanghai, China; A) and incubation time (B) on the fluorescence intensity. Concentration of *Staphylococcus aureus*: 10^8 cfu/mL. V = the volume of SYBR Green I. Error bars indicate the deviation range in triplicate test results.

the recovery of DNA using Si-MNP in pork, which was lower than in milk-powder samples.

To investigate the universal applicability of this protocol further, 80 spiked milk powder and pork samples and 20 negative milk powder and pork samples were tested qualitatively. As shown in Table 2, sensitivities of 100 and 95% were obtained in milk powder and pork samples, respectively. Moreover, the results demonstrated that the protocol had a high specificity (100%) for qualitative detection of *Staph. aureus*.

Optimization of the Detection Conditions

Because the tHDA protocol used the change in fluorescence of SYBR Green I bound to the dsDNA, the concentration of SYBR Green I might have affected the assay performance. To test this possibility, the effect of SYBR Green I concentration on the assay performance was evaluated. As shown in Figure 6A, the fluorescence intensity of SYBR Green I–dsDNA increased when the concentration of SYBR Green I was increased in the range from 5 to 7 μL ; it then plateaued from 7 to 10 μL . Therefore, 7 μL of SYBR Green I was selected for subsequent assays. Similarly, the incubation time with SYBR Green I to detect *Staph. aureus* was optimized. As shown in Figure 6B, the fluorescence intensity increased greatly from 1 to 5 min and leveled off

thereafter. Therefore, the optimized conditions (7 μL of SYBR Green I and 5 min) were selected to achieve a satisfactory signal.

To determine the relationship between amplification time and fluorescence intensity in pure cultures, the tHDA reaction was performed using different amplification times. As shown in Figure 7, relatively good linearity was observed after 60 min of amplification ($R^2 = 0.9970$). Therefore, 60 min was selected for subsequent quantitative assays.

Quantitative Detection Using the Novel Protocol

The relationship between the SYBR Green I fluorescence intensity and the concentration of *Staph. aureus* was investigated further using the optimized conditions. As shown in Figure 8, the data showed a good linearity range between 10^2 and 10^4 cfu/mL of *Staph. aureus*. The fitted regression equation was $F = 0.02225C + 49.29$, where F is the fluorescence intensity and C is the concentration of *Staph. aureus*, with a reliable correlation coefficient ($R^2 = 0.9972$). Interestingly, the data could be fitted to a polynomial curve across a wide dynamic range (data not shown).

Furthermore, recovery studies were performed by spiking samples of milk powder and pork with different concentrations of *Staph. aureus* (ranging from 10^2 to 10^4

Table 2. Evaluation of Si-MNP–tHDA–SYBR Green I assay in spiked milk powder and pork samples¹

Sample	Sample size		Test results	
	Positive	Negative	Sensitivity (%)	Specificity (%)
Milk powder	80	20	100	100
Pork	80	20	95	100

¹Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. SYBR Green I (Invitrogen, Shanghai, China).

Figure 7. Influence of amplification time on the fluorescence intensity (cell counts of *Staphylococcus aureus* ranging from 1×10^2 to 1×10^4 cfu/mL). Color version available online.

cfu/mL) to evaluate the reliability of the quantitative detection system. As shown in Figure 9, the *Staph. aureus* concentrations measured were very close to those added ($P > 0.05$). The results suggested that *Staph. aureus* concentrations from 10^2 to 10^4 cfu/mL could be determined quantitatively using the novel Si-MNP-tHDA-SYBR Green I detection system.

The combination of helicase-dependent isothermal amplification and lateral flow provided an ideal platform for an assay to detect *Staph. aureus* with a detection limit of 2×10^4 cfu/mL in blood culture medium (Goldmeyer et al., 2008); however, this assay

Figure 8. The linear relationship between the fluorescence intensity and the concentrations of *Staphylococcus aureus* (cell counts of *Staph. aureus* ranging from 1×10^2 to 4×10^4 cfu/mL). Error bars indicate the deviation range in triplicate test results. F = fluorescence intensity, C = concentration of *Staph. aureus*.

Figure 9. The recovery of *Staphylococcus aureus* in milk powder and pork samples detected by the Si-MNP-tHDA-SYBR Green I assay. Si-MNP = silica-coated magnetic nanoparticles; tHDA = thermophilic helicase-dependent isothermal amplification. SYBR Green I (Invitrogen, Shanghai, China). Error bars indicated the deviation range in triplicate test results ($P > 0.05$).

could not be used for quantitative analysis. Compared with conventional PCR-based techniques, the novel Si-MNP-tHDA-SYBR Green I method required a shorter sample preparation time, and the detection sensitivity was superior.

CONCLUSIONS

The present study describes the development and optimization of a Si-MNP-based tHDA assay to detect the specific DNA fragments of *Staph. aureus* using SYBR Green I. In the assay, expensive instrumentation was not required, and the results could be detected qualitatively by visual examination under UV lamp or quantitatively using a spectrophotometer. The entire detection time of the protocol was less than 2 h, and its sensitivity and specificity were confirmed using spiked samples. The method shows a great promise for the detection of *Staph. aureus* in food samples. Furthermore, our Si-MNP-based tHDA detection strategy could provide an easy, convenient, and rapid method for sensing a wide range of pathogens with the use of appropriate primers.

ACKNOWLEDGMENTS

This project was sponsored by “Twelfth Five-Year Plan” for the National Science and Technology Support Program (2011BAK10B06, 2011BAK10B01, 2011BAK10B02), Natural Science Foundation (31260363, 31000048), the Ganpo Talent Engineering 555 Project (18000035), the Academic and Technical

Leaders Training Program for Major Subjects of Jiangxi Province (2009-12), and the Research Program of the State Key Laboratory of Food Science and Technology of Nanchang University (SKLF-ZZA-201302, SKLF-TS-200916).

REFERENCES

- Chen, F., X. Ming, X. Chen, M. Gan, B. Wang, F. Xu, and H. Wei. 2014a. Immunochromatographic strip for rapid detection of *Cronobacter* in powdered infant formula in combination with silica-coated magnetic nanoparticles separation and 16S rRNA probe. *Biosens. Bioelectron.* 61:306–313.
- Chen, X., M. Gan, H. Xu, F. Chen, X. Ming, H. Xu, H. Wei, F. Xu, and C. Liu. 2014b. Development of a rapid and sensitive quantum dot-based immunochromatographic strip by double labeling PCR products for detection of *Staphylococcus aureus* in food. *Food Contr.* 46:225–232.
- Doseeva, V., T. Forbes, J. Wolff, Y. Khripin, D. O'Neil, T. Rothmann, and I. Nazarenko. 2011. Multiplex isothermal helicase-dependent amplification assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Diagn. Microbiol. Infect. Dis.* 71:354–365.
- Gilbert, G. L. 2002. Molecular diagnostics in infectious diseases and public health microbiology: Cottage industry to postgenomics. *Trends Mol. Med.* 8:280–287.
- Gill, P., A.-H. Alvandi, H. Abdul-Tehrani, and M. Sadeghizadeh. 2008. Colorimetric detection of *Helicobacter pylori* DNA using isothermal helicase-dependent amplification and gold nanoparticle probes. *Diagn. Microbiol. Infect. Dis.* 62:119–124.
- Gill, P., and A. Ghaemi. 2008. Nucleic acid isothermal amplification technologies—A review. *Nucleosides Nucleotides Nucleic Acids* 27:224–243.
- Goldmeyer, J., H. Li, M. McCormac, S. Cook, C. Stratton, B. Lemieux, H. Kong, W. Tang, and Y. W. Tang. 2008. Identification of *Staphylococcus aureus* and determination of methicillin resistance directly from positive blood cultures by isothermal amplification and a disposable detection device. *J. Clin. Microbiol.* 46:1534–1536.
- Haible, D., S. Kober, and H. Jeske. 2006. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *J. Virol. Methods* 135:9–16.
- Hellyer, T. J., and J. G. Nadeau. 2004. Strand displacement amplification: A versatile tool for molecular diagnostics. *Expert Rev. Mol. Diagn.* 4:251–261.
- Kim, C.-H., M. Khan, D. Morin, W. Hurley, D. Tripathy, M. Kehrli Jr., A. Oluoch, and I. Kakoma. 2001. Optimization of the PCR for detection of *Staphylococcus aureus* nuc gene in bovine milk. *J. Dairy Sci.* 84:74–83.
- Kim, J., and C. J. Easley. 2011. Isothermal DNA amplification in bioanalysis: Strategies and applications. *Bioanalysis* 3:227–239.
- Lazcka, O., F. Campo, and F. X. Munoz. 2007. Pathogen detection: A perspective of traditional methods and biosensors. *Biosens. Bioelectron.* 22:1205–1217.
- Li, M., Y.-L. Wong, L.-L. Jiang, K.-L. Wong, Y.-T. Wong, C. B.-S. Lau, and P.-C. Shaw. 2013. Application of novel loop-mediated isothermal amplification (LAMP) for rapid authentication of the herbal tea ingredient *Hedyotis diffusa* Willd. *Food Chem.* 141:2522–2525.
- Lowy, F. D. 1998. *Staphylococcus aureus* infections. *N. Engl. J. Med.* 339:520–532.
- Mahalanabis, M., J. Do, H. A. L. Muayad, J. Y. Zhang, and C. M. Klapperich. 2010. An integrated disposable device for DNA extraction and helicase dependent amplification. *Biomed. Microdevices* 12:353–359.
- Miao, T., Z. Wang, S. Li, and X. Wang. 2010. Sensitive fluorescent detection of *Staphylococcus aureus* using nanogold linked CdTe nanocrystals as signal amplification labels. *Mikrochim. Acta* 172:431–437.
- Mollasalehi, H., and R. Yazdanparast. 2013. Development and evaluation of a novel nucleic acid sequence-based amplification method using one specific primer and one degenerate primer for simultaneous detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Anal. Chim. Acta* 770:169–174.
- Niessen, L., J. Luo, C. Denschlag, and R. F. Vogel. 2013. The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants. *Food Microbiol.* 36:191–206.
- Omiccioli, E., G. Amagliani, G. Brandi, and M. Magnani. 2009. A new platform for real-time PCR detection of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157 in milk. *Food Microbiol.* 26:615–622.
- Picard, C., J. Fioramonti, A. Francois, T. Robinson, F. Neant, and C. Matuchansky. 2005. Review article: Bifidobacteria as probiotic agents—Physiological effects and clinical benefits. *Aliment. Pharmacol. Ther.* 22:495–512.
- Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—Major pathogens. *Emerg. Infect. Dis.* 17:7–15.
- Vancraeynest, D., F. Haesebrouck, and K. Hermans. 2007. Multiplex PCR assay for the detection of high virulence rabbit *Staphylococcus aureus* strains. *Vet. Microbiol.* 121:368–372.
- Vincent, M., Y. Xu, and H. Kong. 2004. Helicase-dependent isothermal DNA amplification. *EMBO Rep.* 5:795–800.
- Wang, C.-H., K.-Y. Lien, J.-J. Wu, and G.-B. Lee. 2011. A magnetic bead-based assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* by using a microfluidic system with integrated loop-mediated isothermal amplification. *Lab Chip* 11:1521–1531.
- Yang, A. K., H. Lu, S. Y. Wu, H. C. Kwok, H. P. Ho, S. Yu, A. K. Cheung, and S. K. Kong. 2013. Detection of Pantone-Valentine Leukocidin DNA from methicillin-resistant *Staphylococcus aureus* by resistive pulse sensing and loop-mediated isothermal amplification with gold nanoparticles. *Anal. Chim. Acta* 782:46–53.
- Yang, Y., X.-d. Su, Y.-w. Yuan, C.-y. Kang, Y.-j. Li, W. Zhang, and X.-y. Zhong. 2007. Detection of *Staphylococcus aureus* in dairy products by polymerase chain reaction assay. *Agric. Sci. China* 6:857–862.