Saltatory rolling circle amplification for sensitive visual detection of *Staphylococcus aureus* in milk

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ABSTRACT

Monitoring *Staphylococcus aureus* with high sensitivity is very important for ensuring milk quality and food safety. In this study, we used a rapid nucleic acid isothermal amplification method, saltatory rolling circle amplification (SRCA), for the detection of *Staph. aureus* in milk. The results of the SRCA method can be assessed visually by the presence of white precipitate or by fluorescence measurement. Thirteen *Staph. aureus* strains and 31 non-*Staph. aureus* strains were used to evaluate the specificity of SRCA. The method exhibited excellent detection of *Staph. aureus* genomic DNA at a concentration of 7.8×10^1 fg/ μ L when assessed by visible precipitate, and at 7.8×10^0 fg/ μ L when detected by fluorescence after addition of the fluorochrome SYBR Green I. In artificially inoculated milk, the detection limits of SRCA were 5.6×10^2 cfu/mL by precipitate and 5.6×10^1 cfu/mL by fluorescence, respectively. Compared with conventional PCR approaches, the SRCA assay achieved at least 100-fold higher sensitivity. Moreover, the sensitivity, specificity, and accuracy of the SRCA-based system were calculated to be 100.00, 97.73, and 97.78%, respectively. These results indicate that SRCA has potential application as a sensitive and visual technique for the detection of *Staph. aureus* in milk.

Key words: saltatory rolling circle amplification (SRCA), *Staphylococcus aureus*, visualization, detection

INTRODUCTION

Bovine mastitis is an important disease in the dairy industry worldwide, resulting in economic losses and decreased quality and production of milk (Silva et al., 2013; Zhang et al., 2015). Multiple pathogens are

known to cause bovine mastitis (Fischer-Tenhagen et al., 2018); in particular, *Staphylococcus aureus* is one of most common pathogens in dairy animals (Schlegelová et al., 2003). *Staphylococcus aureus* poses a potential public health threat because of the presence of staphylococcal enterotoxins, which can lead to food poisoning, life-threatening endocarditis, septicemia, vomiting, and diarrhea (Shahdordizadeh et al., 2017). Consequently, a rapid and sensitive method for the detection of *Staph. aureus* in milk is essential for food safety and public health.

Conventional culture-based methods—the gold standard for the detection of *Staph. aureus*—are inexpensive and reliable; however, these techniques suffer from some shortcomings, including being laborious and time consuming (Dong et al., 2018). Methods based on PCR, such as real-time PCR (Botaro et al., 2013), address these weaknesses because of their specificity and sensitivity but require skilled operation and costly laboratory facilities. Recently, a nucleic acid isothermal amplification method, loop-mediated isothermal amplification (LAMP; Nawattanapaboon et al., 2015), was developed for the detection of pathogens. However, 4 to 6 primers are required for LAMP, which could lead to the interaction of primers in the reaction system. Furthermore, the primer design of LAMP is intricate and expensive (Suleman et al., 2016; Yang et al., 2016). Rolling circle amplification (RCA; Hao et al., 2017), that is, synthesizing a quantity of DNA fragments from low initial DNA concentrations, has advantages for the detection of pathogens. Nevertheless, to amplify linear DNA, the circularization of padlock probes and ligase are indispensable, making RCA complicated.

Saltatory rolling circle amplification (SRCA) is a new and interesting type of nucleic acid isothermal amplification technique that depends on *Bst* DNA polymerase, which can synthesize DNA independent of a template (Zyrina et al., 2007). The schematic diagram of the SRCA amplification principle is shown in Figure 1. After a pre-denaturation step, the reverse primer (RVP) combines with the complementary sequence

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in the target single-strand DNA (ssDNA), and the reaction is initiated (step 1). Later, the forward primer (FWP) is bound to the complementary sequence of the newborn DNA strand, producing a double-stranded DNA (step 2). Next, RVP binds to the complementary sequence of the nascent DNA strand and DNA is synthesized at the 3' end. When the primer binds to the 3' end of the template, the single DNA strand is displaced and a gapped secondary structure is formed (steps 3, 4). The FWP binds to complementary region of the secondary structure and extends from the 3' end to the 5' end (step 5). When FWP extends to the gap, it can continue to expand by adding several bases with the action of *Bst* DNA polymerase; *Bst* continues to add nucleotides when it extends to the 5' end of the template (step 6). Subsequently, *Bst* continues to produce new DNA strands and previous DNA strands are replaced and the first-round synthesis amplification has been accomplished (step 7). Then, the extended products of FWP and combined FWP are displaced and a long ssDNA continues to generate. With the extending ssDNA, an increasing number of RVP binding sites are exposed. Then, RVP binds to the corresponding binding sites of new ssDNA (step 8). Each RVP will continue to extend and displace the amplified products. Finally, many tandem repeat units and ssDNA molecules of different lengths are generated (step 9). The FWP binding sites of tandem repeat unit ssDNA are effective for FWP binding and extending (step 10), sequentially producing several linear, double-stranded, tandem repeat unit DNA sequences (containing the target sequence and bases added, step 11).

Different from the LAMP method that was originally published (Notomi et al., 2000), SRCA is simpler, in that it requires only 2 primers, and cheaper, about 50% of the cost of LAMP. Moreover, the amplification results of SRCA can be ascertained by sequencing. Compared with the RCA method originally published (Lizardi et al., 1998), the need for padlock probes and ligase is averted and the circularization of probes is unnecessary. Under the circumstances of amplification of linear DNA, the operation of SRCA is easy and takes 3 h less than RCA. The results can be visualized by adding a fluorochrome or observing the white precipitate. In addition, SRCA does not require thermal cycling and just a simple temperature controller is needed, such as a water bath or a heating block.

Considering the aspects described above, the objective of this study was, for the first time, to detect *Staph. aureus* strains in milk by targeting the factors essential for methicillin-resistance (*femA*) gene using the SRCA method; *femA* encodes the factor necessary for methicillin resistance and has high specificity (Li et al., 2008). The SRCA method could efficiently negate

the need for expensive instruments and time-consuming electrophoresis, and results could be assessed via visible precipitate or fluorescence. The effectiveness of the developed method was validated by calculating specificity, sensitivity, and detection limits. Finally, we detected *Staph. aureus* in real milk samples by the SRCA method.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

In total, 44 different strains were adopted for specificity evaluation (Table 1): 13 *Staph. aureus* strains and 31 non-*Staph. aureus* strains. *Staphylococcus aureus* ATCC 43300 was used for the determination of sensitivity and detection limit. All strains were cultured in nutrient broth (Beijing Land Bridge Technology Co. Ltd., Beijing, China) overnight at 37°C for 12 h.

DNA Extraction

The total DNA of these 44 strains was extracted from fresh overnight-cultured bacteria solution using the EasyPure Bacteria Genomic DNA kit (Takara Biotechnology Co. Ltd., Dalian, China). The DNA samples were stored at -20°C until analyzed and quantified using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) by measuring absorbance (A) at wavelengths of 260 and 280 nm.

Primer Design

The target gene used for SRCA was *femA* (GenBank accession No. DQ352467.1). The principle of SRCA primer design is similar to that of the PCR method, and is based on factors such as the GC content, mismatch rate, and so on. The DNAMAN software (Beijing Genomics Institute, Beijing, China) was used to design the primers, which are listed in Table 2.

SRCA and PCR Conditions

The total volume of the SRCA reaction system was 20 µL, which contained DNA template (1.0 µL), dNTP mixture (2 mM), 10× ThermoPol Buffer (2 µL), MgSO₄ (3 mM), each primer (0.5 µM), and *Bst* DNA polymerase (8 U), with deionized water added to make a final volume of 20 µL. All of the samples were incubated at 62°C for 60 min and then at 80°C for 5 min to end the reaction. The SRCA reaction required a pre-denaturation step (94°C for 3 min) and an ice-bath (2 min) before the reaction.

Figure 1. The schematic presentation of the developed saltatory rolling circle amplification (SRCA) method. The blue and pink arrow lines show the forward (FWP) and reverse (RVP) primers, respectively. The black line indicates other sequences in the target template. The green and red lines represent the binding regions of FWP and RVP, respectively. The purple line reflects other sequences of reverse complement of target DNA template. The yellow line indicates the nucleotides added between the 3' and 5' ends of the template, and its complementary sequence is highlighted in orange. The ellipsis (- - -) reveals omitted steps and products. The order of steps (1) to (11) is shown by black arrows. Different lengths of amplification products are shown as double lines of various colors in the last step.

The PCR reaction mixture contained DNA template (1 μ L), each primer (0.4 μ M), 2 $\times$ EasyTaq PCR Super-Mix (11 μ L; TransGen Biotech, Beijing, China), and deionized water to 25 μ L. Thermal cycling conditions were as follows: denaturation at 95°C for 5 min, followed by 29 cycles (95°C for 30 s, 58°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 45 s (Yang et al., 2016).

Restriction Enzyme Digestion and Sequence Analysis of the SRCA Products

To confirm the specificity of SRCA, the amplicons were digested at 37°C for 1 h using *Hind*III restriction

enzyme (Takara Biotechnology Co. Ltd.) following the instructions. Then, the digested products were analyzed by electrophoresis. Four fragments were cloned for sequencing analysis by Beijing Genomics Institute (Beijing, China).

Sensitivity of the SRCA Assay for *Staph. aureus*

To determine the sensitivity of SRCA, 10-fold serial dilutions of *Staph. aureus* (ATCC 43300) with sterile saline were prepared at DNA concentrations ranging from 7.8×10^7 to 7.8×10^{-1} fg/ μ L. We compared SRCA sensitivity with that of the traditional PCR method. The sensitivity of SRCA was checked by electrophore-

Table 1. *Staphylococcus aureus* and non-*Staph. aureus* strains and their saltatory rolling circle amplification (SRCA) results (positive or negative)

No.	Strain	Source ¹	SRCA result
1	<i>Staph. aureus</i>	ATCC 43300	+
2	<i>Staph. aureus</i>	ATCC 25923	+
3	<i>Staph. aureus</i>	CICC 21600	+
4	<i>Staph. aureus</i>	CICC 21648	+
5	<i>Staph. aureus</i>	CMCC 26073	+
6	<i>Staph. aureus</i>	CMCC 26003	+
7	<i>Staph. aureus</i>	LP	+
8	<i>Staph. aureus</i>	LP	+
9	<i>Staph. aureus</i>	LP	+
10	<i>Staph. aureus</i>	LP	+
11	<i>Staph. aureus</i>	LP	+
12	<i>Staph. aureus</i>	LP	+
13	<i>Staph. aureus</i>	LP	+
14	<i>Staphylococcus epidermidis</i>	CICC 23664	–
15	<i>Staphylococcus xylosus</i>	CICC 20237	–
16	<i>Staphylococcus xylosus</i>	CICC 22943	–
17	<i>Pediococcus acidilactici</i>	CICC 20719	–
18	<i>Streptococcus pneumoniae</i>	CICC 10913	–
19	<i>Streptococcus pyogenes</i>	CICC 10373	–
20	<i>Micrococcus luteus</i>	LP	–
21	<i>Listeria ivanovii</i>	CICC 21663	–
22	<i>Listeria monocytogenes</i>	CICC 21633	–
23	<i>Escherichia coli</i>	ATCC 25922	–
24	<i>Escherichia coli</i>	CMCC 44103	–
25	<i>Escherichia coli</i>	CICC 10372	–
26	<i>Escherichia coli</i> O157:H7	CICC 21530	–
27	Diarrheogenic <i>Escherichia coli</i>	CICC 10411	–
28	<i>Salmonella enteritidis</i>	CMCC 50041	–
29	<i>Salmonella typhimurium</i>	CMCC 50115	–
30	<i>Salmonella paratyphi</i> -A	CMCC 50001	–
31	<i>Salmonella paratyphi</i> -B	CICC 21495	–
32	<i>Shigella flexneri</i>	CMCC 51571	–
33	<i>Shigella sonnei</i>	CMCC 51334	–
34	<i>Shigella boydii</i>	CMCC 51522	–
35	<i>Shigella dysenteriae</i>	CICC 23829	–
36	<i>Alicyclobacillus acidoterrestris</i>	ATCC 49025	–
37	<i>Vibrio parahaemolyticus</i>	ATCC 17802	–
38	<i>Vibrio alginolyticus</i>	CICC 21664	–
39	<i>Cronobacter sakazakii</i>	ATCC 29544	–
40	<i>Acetobacter pasteurianus</i>	ATCC 33445	–
41	<i>Pseudomonas aeruginosa</i>	CICC 21636	–
42	<i>Clostridium sordellii</i>	CICC 22950	–
43	<i>Proteus bacillus vulgaris</i>	CMCC 49027	–
44	<i>Bacillus cereus</i>	CMCC 63302	–

¹ATCC = American Type Culture Collection (Manassas, VA); CICC = China Center of Industrial Culture Collection (Beijing, China); CMCC = National Center for Medical Culture Collections (Beijing, China); LP = laboratory preserved.

sis and results were visualized by observing the white precipitate or by adding fluorochrome SYBR Green I (1 μ L of 1,000 $\times$; Takara Biotechnology Co. Ltd.).

SRCA Assays of Milk Spiked with *Staph. aureus*

To study the performance of SRCA, milk samples purchased from local supermarkets (Baoding, China) were first tested by a traditional detection method (CFDA, 2016) and a PCR method to confirm the absence of *Staph. aureus* strains. *Staphylococcus aureus*

Table 2. Saltatory rolling circle amplification (SRCA) and PCR primers

Primer	Sequence	Melting temperature (°C)
Forward	5'-AGAGTTTGGTGCATTTACAG-3'	56.9
Reverse	5'-AGTGGCCAACAGTTTGCGTG-3'	58.0

was then used to artificially contaminate the milk samples. Briefly, 25 mL of milk sample was homogenized with 225 mL of sterile 7.5% sodium chloride broth to produce a homogenate. Then, 1 mL of the overnight-cultured *Staph. aureus* bacteria solution was serially diluted in 9 mL of the homogenate. In parallel, 1 mL of overnight-cultured *Staph. aureus* was serially diluted in 9 mL of sterile normal saline for plate counts. The initial concentration of *Staph. aureus* was 5.6×10^9 cfu/mL, and concentrations of *Staph. aureus* in milk homogenate ranged from 5.6×10^8 to 5.6×10^0 cfu/mL. Subsequently, 1 mL of each inoculated homogenate was used for DNA extraction. An uninoculated milk sample was used as the negative control. The extracted genomic DNA was used to determine detection limits of SRCA and PCR. The SRCA products were also evaluated by 2% agarose gel electrophoresis and by production of white precipitate and fluorescence.

Detection of *Staph. aureus* in Milk Samples

A total of 90 milk samples were collected from the supermarkets (Baoding, China). The sensitivity, specificity, and accuracy of the SRCA system were determined by comparison with detection of *Staph. aureus* by ISO methods (ISO, 2016a,b). Statistical significance was defined as $P < 0.05$. The equations to calculate sensitivity, specificity, and accuracy are as follows:

$$\text{Sensitivity: } (T_{\text{pos}})/(T_{\text{pos}} + F_{\text{neg}}) \times 100\%,$$

$$\text{Specificity: } (T_{\text{neg}})/(T_{\text{neg}} + F_{\text{pos}}) \times 100\%,$$

$$\text{Accuracy: } (T_{\text{pos}} + T_{\text{neg}})/(T_{\text{pos}} + T_{\text{neg}} + F_{\text{pos}} + F_{\text{neg}}) \times 100\%,$$

where T_{pos} and T_{neg} are true positive and true negative samples, confirmed by both ISO and SRCA methods, and F_{pos} and F_{neg} are false positive and false negative samples, confirmed only by the ISO or SRCA method, respectively. The experiments were performed over a short period of time in triplicate, each with 3 repeats under the same conditions (method, test samples, laboratory, operator, and equipment) to determine the sensitivity and detection limit.

RESULTS AND DISCUSSION

Detection of *Staph. aureus* by SRCA

In this study, we adopted the *femA* gene of *Staph. aureus* for primer design. The ladder-like bands appeared as a positive reaction by 2% agarose gel electrophoresis,

and were not observed in the negative control (panel A; Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-16724>). The white precipitate was clearly visible by the naked eye (tube 1), and no white precipitate was found in the negative control (tube 2, Supplemental Figure S1B). As shown in Figure S1C, tube 1 showed a bright green color under daylight, but tube 2 remained

Figure 2. Restriction enzyme and sequence analysis results of saltatory rolling circle amplification (SRCA) products from *Staphylococcus aureus*. (A) Lane M = 100-bp DNA ladder; lane 1 = SRCA amplification products from *Staph. aureus*; lane 2 = PCR amplification products from *Staph. aureus*; lane 3 = digestion fragments from SRCA amplification products; (B) lane M = 100-bp DNA ladder; lane 1 = SRCA amplification products from *Staph. aureus* by 2% agarose gel electrophoresis; (C) sequencing results of the fragments annotated as 1 to 4 in (B). Sequences highlighted in blue and green correspond to the forward primer (FWP) and the reverse complementary sequence of the reverse primer (RVP), respectively. The sequence beginning from the blue highlight to green highlight indicates the target sequence of 62 bp. The sequences in red font are the added nucleotides between the tandem-repeated targeting DNA.

its original orange color. Compared with the traditional rolling circle amplification method, there is no need for padlock probes and ligase in SRCA, which simplified operation and reduced the time needed. Only 2 primers are required in SRCA, whereas 4 to 6 primers are needed in LAMP, increasing the expenditure and the possibility of interaction among primers. In summary, SRCA is an economical method with the advantage that results can be visualized without specialized equipment.

Validation of the SRCA Products

To validate the positive SRCA products, amplicons were digested using restriction enzyme *Hind*III, forming 3 bands at 106, 96, and 72 bp. Only 2 bands are observed in Figure 2A; the upper bands (96 and 106 bp) are close in size and not well separated. Four bands in Figure 2B were sequenced to further validate the SRCA products. The results in Figure 2C showed that the target fragments were 100% homologous to the *femA* gene, and the products were composed of tandem-repeated units (containing the target sequence and bases added). These experimental results showed that the SRCA amplification products matched well with the *femA* target gene in *Staph. aureus*.

Specificity

The *femA* gene exists in all *Staph. aureus* strains and has been reported to be specific to *Staph. aureus* (Zhao et al., 2013); it is not found in other *Staphylococcus* species (Sun et al., 2014). Recently, a PCR assay (McGann et al., 2013) and LAMP method (Xu et al., 2012) targeting *femA* for the detection of *Staph. aureus* have been reported, confirming the high specificity of *femA*. Therefore, we also adopted *femA* as the specificity assay in this study.

The specificity of the SRCA assay was assessed using 13 *Staph. aureus* strains and 31 non-*Staph. aureus* strains (Table 1). As expected, the typical bands on 2% agarose gel electrophoresis were clearly observed in 13 *Staph. aureus* and were absent in the 31 non-*Staph. aureus* strains (Figure 3), suggesting that the SRCA method had high specificity for *Staph. aureus*.

Sensitivity of SRCA Assay

The serially diluted DNA extracted from *Staph. aureus* was used as a template to analyze the sensitivity of the SRCA assay. As concentrations decreased from 7.8×10^7 to 7.8×10^1 fg/ μ L, the ladder-like bands increased in size and the white precipitate was obvious. No bands or white precipitate was observed at concentrations of 7.8×10^0 and 7.8×10^{-1} fg/ μ L (Figure 4A and B).

Therefore, the sensitivity of SRCA was 7.8×10^1 fg/ μ L by electrophoresis and by visualization of white precipitate. As shown in Figure 4C, the PCR products were visible at concentrations from 7.8×10^7 to 7.8×10^3 fg/ μ L, but not for concentrations from 7.8×10^2 to 7.8×10^{-1} fg/ μ L. Additionally, by adding fluorochrome SYBR Green I, an obvious green color could be seen as the concentration decreased from 7.8×10^7 to 7.8×10^0 fg/ μ L, but no color change was observed at 7.8×10^{-1} fg/ μ L (Figure 4D). Therefore, the sensitivity of SRCA was 7.8×10^0 fg/ μ L by fluorescence, which was 10-fold higher than that of electrophoresis or visualization via white precipitate, and 1,000-fold greater than that of the conventional PCR method, respectively.

Figure 3. Specificity assay. M = 100-bp DNA ladder; N = negative control; P = positive control; lanes 1–14 (A) corresponding to strains listed in Table 1; lanes 15–28 (B) as listed in Table 1; lanes 29–44 (C) as listed in Table 1.

Of particular note, the LAMP assay has been widely applied for the detection of pathogens with high sensitivity. According to a previous report (Sheet et al., 2016), as little as 0.26 pg of *Staph. aureus* DNA per reaction could be detected by using the LAMP method. A group of LAMP primers was designed for the analysis of *Staph. aureus* in clinical blood plaque samples and the sensitivity was 1.47 pg/ μ L (Wang et al., 2015). The sensitivities of our SRCA assay were 7.8×10^1 fg/ μ L for detection by electrophoresis and white precipitate; the sensitivity of detection by visualization with fluorescence was 7.8×10^0 fg/ μ L, which is comparable

to that of LAMP-based techniques. Overall, the experimental results showed that the SRCA assay had excellent sensitivity.

Detection of *Staph. aureus* in Spiked Milk

We confirmed the detection of *Staph. aureus* in spiked milk using the SRCA method. The SRCA detection limits of *Staph. aureus* in spiked milk by electrophoresis and visualization of white precipitate were both 5.6×10^2 cfu/mL (panels A and B, Supplemental Figure S2; <https://doi.org/10.3168/jds.2019-16724>). The SRCA

Figure 4. Sensitivities of (A) saltatory rolling circle amplification (SRCA) assay on 2% agarose gel electrophoresis; (B) SRCA assay determined visually by white precipitate; (C) conventional PCR method by gel electrophoresis; (D) visual SRCA with fluorescence. M = 100-bp DNA ladder; lane 1 = negative control; lanes 2–10 = concentrations of template DNA ranging from 7.8×10^7 to 7.8×10^{-1} fg/ μ L.

method could detect *Staph. aureus* in spiked milk at 5.6×10^1 cfu/mL when fluorochrome SYBR Green I was added (Figure S2C). By comparison, the detection limit of conventional PCR for *Staph. aureus* was 5.6×10^4 cfu/mL (Figure S2D). The detection limit of a multiplex PCR assay was found to be as low as 10^3 cfu/mL (Yu et al., 2015). In brief, the detection limits of SRCA by electrophoresis and white precipitate were 10- or 100-fold better than that of the PCR-based assays mentioned above. The detection limits of visualization by fluorescence were 100-fold or 1,000-fold better than those of PCR-based assays.

The minimum infective dose of staphylococcal enterotoxins is 1 µg, and enterotoxins are produced when *Staph. aureus* counts in foods exceed 10^5 cfu/mL or cfu/g (Ahmed et al., 2019). According to the US Food and Drug Administration regulation, the count of *Staph. aureus* in raw milk or other dairy products needs to be less than 10^4 cfu/mL or cfu/g, which is similar to that in other countries, such as Australia and New Zealand (Yu et al., 2016). The detection limits of *Staph. aureus* in artificially contaminated milk by SRCA were 5.6×10^2 cfu/mL by electrophoresis and visualization of white precipitate or 5.6×10^1 cfu/mL by fluorescence, both of which were lower than 10^4 cfu/mL, indicating that the SRCA method could be used to detect *Staph. aureus* in milk and possibly other food products.

Detection of *Staph. aureus* in Real Milk Samples

The sensitivity, specificity, and accuracy of the SRAC assay are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-16724>). In this study, 4 milk samples were found to be positive by SRCA, whereas only 2 samples positive for *Staph. aureus* were detected by the ISO method, perhaps reflecting the fact that the sensitivity of SRCA is much higher than that of the ISO method. In addition, the DNA of viable but nonculturable (VBNC) or dead bacteria can be amplified by the SRCA method. By comparison with the ISO method as reference, the SRCA method had 100.00% sensitivity, 97.73% specificity, and 97.78% accuracy.

CONCLUSIONS

In this study, we developed a rapid SRCA technique for the detection of *Staph. aureus* in milk, with sensitivity of 7.8×10^0 fg/µL in pure culture and a detection limit of 5.6×10^1 cfu/mL. In particular, SRCA needs only 2 primers to complete the amplification, and a positive result can be visualized by the naked eye as a white precipitate or fluorescence. To the best of our knowledge, this is the first report to use the SRCA

method to detect *Staph. aureus* in milk. The developed method is rapid and sensitive, and is expected to provide a unique opportunity to detect *Staph. aureus* in various food samples.

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