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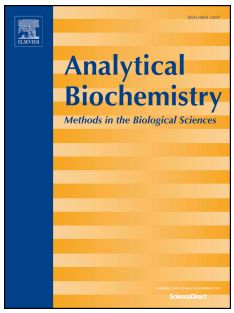

An aptamer biosensor based dual signal amplification system for the detection of *salmonella typhimurium*

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PII: S0003-2697(20)30582-0

DOI: https://doi.org/10.1016/j.ab.2020.114050

Reference: YABIO 114050

To appear in: *Analytical Biochemistry*

Received Date: 10 June 2020

Revised Date: 14 November 2020

Accepted Date: 24 November 2020

Please cite this article as: A. Li, P. Zuo, B.-C. Ye, An aptamer biosensor based dual signal amplification system for the detection of *salmonella typhimurium*, *Analytical Biochemistry*, https://doi.org/10.1016/j.ab.2020.114050.

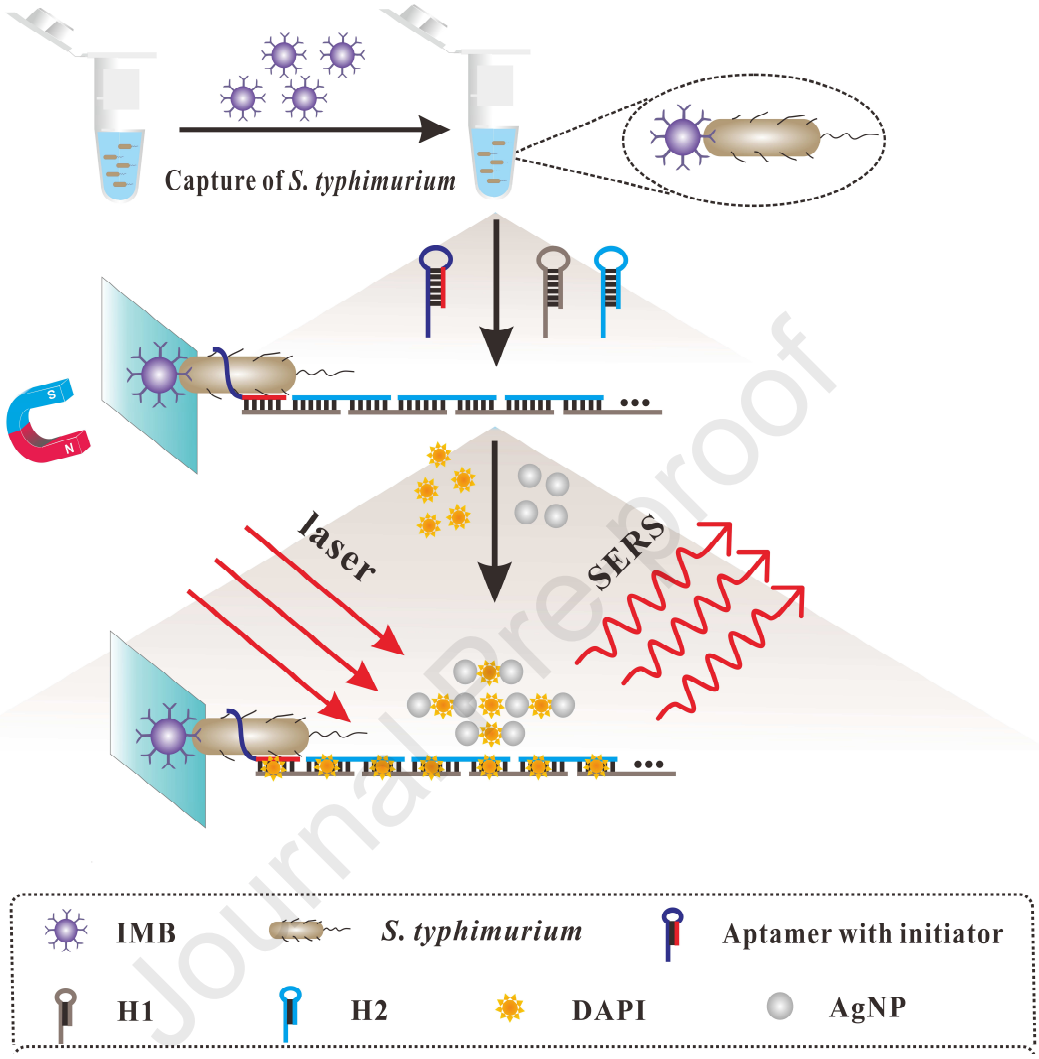
CRediT authorship contribution statement

Ao Li: Literature research, experiments, data collection and analysis, manuscript writing.

Peng Zuo: Conceptualization, methodology, strategy and Data curation, manuscript review and editing.

Bang-Ce Ye: Resources, funding, project administration and supervision.

Graphical Abstract



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An aptamer biosensor based dual signal amplification system for the detection of *salmonella typhimurium*

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Abstract: *Salmonella*, a typical foodborne pathogen, always seriously threatens the health and even life of both humans and animals. However, highly sensitive and fast quantitative methods for its detection are remaining to be challenged. Herein, we presented an efficient method with dual signal amplification strategy by combining immune hybridization chain reaction (HCR) with surface enhanced Raman scattering (SERS) to high sensitively detect *Salmonella typhimurium* in food. After sample preparation, *S. typhimurium* were specifically captured by immunomagnetic beads (IMBs), then aptamers and hairpin-probes were added to trigger HCR to form nicked dsDNA, finally 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) was incubated with HCR products and then the whole system was mixed with AgNP colloid to detect the SERS intensity at 1610 cm^{-1}. As a result, a good linear relationship was achieved between SERS intensities and corresponding concentrations of *S. typhimurium* ranging from 10 to 10^5 CFU/mL, with a limit of detection (LOD) of 6 CFU/mL in 3.5 h. The proposed method has been successfully applied to capture and detect the *S. typhimurium* in spiked milk samples, and the results were consistent with those of the traditional plate counting methods. The method, with combination of HCR and SERS, achieves double amplification of the detection signal and significantly improves the detection sensitivity of *S. typhimurium*. And also shows good application potential for the highly sensitive detection of other contaminants in food.

Keywords: S. typhimurium; HCR; SERS; dual signal amplification; food safety detection

1. Introduction

Salmonella has attracted increasing attention in the last few decades, especially *Salmonella typhimurium*, as it is considered to be one of the most dangerous foodborne pathogens to humans and animals [1, 2]. Foods contaminated by *S. typhimurium* hardly show obvious changes in sensory properties, so they are always prone to be eaten by humans and livestock by mistake, causing food-borne diseases such as gastroenteritis [3]. Usually, ingestion of more than 10^5 CFU *Salmonella* makes normal people infected, while 15 to 20 CFU for immunocompromised or highly susceptible people [4, 5]. Food safety incidents caused by *Salmonella* are still emerging frequently worldwide. Therefore, it is of great importance to develop new methods for highly sensitive detection of *Salmonella* to ensure food safety.

Various analytical methods for the detection of *S. typhimurium* have been developed especially in the past few decades. Although regarded as the gold standard, the traditional detection method is time-consuming and labor-intensive, which makes it impossible to meet the urgent need for immediate detection. In recent years, great improvements have been made in sensitivity and detection time for the analytical methods based on immunology or molecular biology, such as ELISA [6, 7], PCR [8, 9] and loop-mediated isothermal amplification (LAMP) [10]. However, either need complex preparation processes or false positives problem may occur in these methods sometimes. Therefore, the specificity of the detection needs to be improved. Aptamer is usually an oligonucleotide fragment obtained from a library of nucleic acid molecules using systematic evolution of ligands by exponential enrichment (SELEX). It can fold into specific spatial structure and specifically bind to the target [11-13]. Comparing to antibody, aptamer has advantages of stability, inexpensiveness, easy synthesis and chemical modification [14, 15]. Moreover, there is great potential for it to be designed as a structural aptasensor for specific analysis.

Hybridization chain reaction (HCR), as an enzyme-free isothermal nucleic acid amplification technology, has been gradually applied into biosensing fields, such as DNA and RNA detection [16, 17], proteins detection [18] and cells detection [19] due to its superiorities of good amplification ability, easy operation and low cost. When aptamer is modified with an initiator, it can not only specifically bind to its target, but also exposes the initiator and thus touches off HCR. Furthermore, an effective means of signal conversion and output is necessary. Being as a useful analytical tool, surface-enhanced Raman scattering (SERS) has been applied in many fields [20-22] due to its excellent fingerprint recognition and powerful signal amplification. SERS also shows huge prospects for foodborne pathogenic bacteria detection, Duan et al. developed a SERS-based aptasensor to quantitatively detect for *S. typhimurium* with a LOD of 15 CFU/mL[23]; subsequently, similar approaches based on SERS have also been developed and the detection sensitivity has been improved significantly[24, 25]. When HCR combines with SERS for the detection of foodborne pathogens, sensitivity of detection may be further improved.

In order to highly sensitive detect *S. typhimurium*, we developed an efficient method with dual signal amplification strategies by combining HCR with SERS. *S. typhimurium* was first captured by prepared IMBs. Then the aptamers modified with an initiator were utilized to specifically bind to *S. typhimurium* and trigger HCR as signal amplification. Subsequently, DAPIs that had not reacted with HCR products could absorb between the AgNP substrates due to the electrostatic interaction, forming a large number of hot spots, and emitting intensive SERS signals as signal conversion and amplification. Finally, the amounts of *S. typhimurium* can be quantitatively detected by the measured SERS signals. In this combination strategy, dual signal cascade amplification increases detection sensitivity, and aptamer ensures specificity of detection and ease of sample preparation

2. Materials and methods

2.1 Reagents and materials

Dynabeads MyOne™ carboxylic magnetic beads were

purchased from Thermo Fisher Scientific Co., Ltd. (Waltham, MA). MES hydrate was obtained from Acros Organics (Morris Plains, NJ) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was purchased from TCI Co., Ltd. (Shanghai, China). Phosphate buffer solution (PBS, 10×) were purchased from Solarbio Science and Technology Co., Ltd (Beijing, China) and Tween 20 was purchased from Aladdin Inc. (Shanghai, China). Bovine Serum Albumin (BSA) was purchased from Sigma Aldrich, Inc. (Saint Louis, MO). TMB solution was purchased from Beyotime Biotechnology Co. Ltd (Shanghai, China). Rabbit polyclonal antibody to *Salmonella* was purchased from Abcam (Shanghai, China) and Goat anti-Rabbit IgG (H+L) was purchased from YEASEN Biotech Co., Ltd. (Shanghai, China). 4',6-Diamidino-2-phenylindoledihydrochloride（DAPI）was purchased from Dibo Biotechnology Co., Ltd. (Shanghai, China). Silver nitrate was purchased from Chemical reagents Co., Ltd. (Shanghai, China) and trisodium citrate was purchased from Lingfeng chemical reagents Co., Ltd. (Shanghai, China). Almost all kinds of solutions unless specifically stated were prepared using deionized water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ·cm. Oligonucleotides[26, 27] shown in Table S1 were synthesized by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The strains and types are as follows: *Salmonella typhimurium* ASI 1174, *Escherichia coli* ATCC 35128 (*E. coli*), *Staphylococcus aureus* ATCC 12600 (*S. aureus*), and *Mycobacterium smegmatis* CGMCC 10562 (*M. smegmatis*).

2.2 Instrumentation

Bacteria were cultured in a HZ-9211K constant temperature oscillator (Hualida Experimental Equipment Co., Ltd., Jiangsu, China). The preparation of nanosilver was carried out on a collecting thermostatic heating magnetic stirrer (DF-101S, Yuhua Instrument Co., Ltd., Henan, China). Agarose gel electrophoresis was performed on an electrophoresis apparatus (DYY-6C, Liuyi Biotechnology Co., Ltd., Beijing, China) and the gels were visualized under a BioRad Molecular imager (Tanon-1600, China) and Gel Image System (Tanon, China). UV-vis absorption spectra were measured by a microplate reader (Bio-Tek Instrument, Winooski, VT) using a transparent 384-well microplate (Corning Inc., NY) and the determination of Raman spectra was carried out by Raman spectrometer (785nm, B&W Tek, Delaware). The particle size distribution of AgNPs was determined by the Muti-angle Dynamic Light Scattering instrument (Dynapro NanoStar, Wyatt).

2.3 Preparation of immunomagnetic beads

20 μL carboxyl magnetic beads were washed for three times by 200 μL MES solution (15 mM, pH 6) on the magnetic frame. Subsequently, 200 μL freshly prepared EDC solution (10 mg/mL) was added to resuspend the magnetic beads for 30 min at room temperature to activate the carboxyl group on the surface of the magnetic beads, and then 200 μL rabbit polyclonal antibody against *S. typhimurium* (40 mg/mL) was added into the experimental group, while 200 μL PBS in the control group. After vibrating at room temperature for 3 h, the solution was washed twice with 200 μL PBST (0.1% Tween 20 in 1×PBS solution), and finally 800 μL blocking solution (1% BSA in 1×PBS solution) was added. After conjugation, the antibody modified magnetic beads were stored at 4 ℃. In order to verify whether the antibody had coupled with magnetic beads successfully, two experimental groups (1 and 2) and one control group were tested as follow: firstly, 100 μL previously prepared samples were washed for three times with 200 μL PBST, and then 25 μL goat anti-rabbit antibody (0.4 mg/mL) was added. The reaction was carried out for 1 h at room temperature and then washed three times with PBST, after that, 200 μL TMB coloring solution was added and reacted for 1 min, then 50 μL dilute sulfuric acid (2 M) was added to terminate the reaction. Finally, the absorbance was measured on a microplate reader.

2.4 IMBs capture *S. typhimurium*

S. typhimurium were inoculated into LB liquid medium and cultured at 37 ° C overnight in the constant temperature oscillator. After fully mixing, 1 mL bacterial solution was taken and centrifuged at 8000 rpm for 6 min, then remove the supernatant and wash the precipitates with 1 mL PBS buffer; after repeating the operation twice, the precipitate was dispersed in 1 mL PBS buffer and the OD_{600} value was measured. Subsequently, gradient concentrations of the bacterial solution from 10 to 10^5 CFU/mL were diluted according to the OD_{600} standard curve of *S. typhimurium*. At the same time, the preservation solution of prepared IMBs was dispensed in 200 μL per tube and washed three times with PBST. Finally, 1 mL bacterial solution of each gradient was mixed with IMBs and shaken at room temperature for 40 min to form IMB-*S. typhimurium* conjugates.

2.5 Signal amplification by HCR

Stock solutions of H1 and H2 were previously heated to 95 °C for 2 min and then cooled to room temperature before use. In order to obtain the optimal reaction conditions for HCR, temperature (room temperature, 37 °C and 42 °C) and reaction time (0.5 h, 1 h and 2 h) were chosen to improve the extension efficiency of dsDNA, and the results were verified by 2% agarose gel electrophoresis with the condition that electrophoresis was carried out for 15 min at the voltage of 100 v. Under the optimized condithion, 190 μL Tris-HCl buffer (20 mM Tris, 0.5 M sodium chloride, pH 7.8) and 10 μL annealed aptamer (5 μM) were successively added to the IMB-*S. typhimurium* conjugate. After shaking for 50 minutes at 37 ℃, the conjugate was washed with PBST for three times. After that, 160 μL SSC buffer (120 mM sodium citrate, 1.2 M sodium chloride, pH 7), 20 μL H1 (10 μM) and 20 μL H2 (10 μM) solutions were successively added to the IMB - *S. typhimurium*-aptamer conjugate and then shook at 37 ° C for 1 h. After washed three times with PBST, 100 μL sterile water was added and it's ready to be tested.

2.6 Signal amplification and conversion by SERS

The preparation of nano-silver was synthesized according to a modified conventional classical method [28]. Briefly, 163 mL of deionized water was poured into a three-necked flask and heated to 99 ° C on a collecting thermostatic heating magnetic stirrer. Then, 1 mL of 30 mg/mL silver nitrate solution and 3 mL freshly prepared 1% sodium citrate solution were added rapidly. The solution gradually changed from colorless to pale yellow after about 20 min. Subsequently, the temperature was lowered to 90 ° C and the reflux reaction was continued for 1 hour. Finally, the colloid turned grayish green and cooled to room temperature and then stored in the dark at 4 ° C.

The conditions for SERS detection were set as follows: the wavelength of the laser was 785 nm, the laser power was 100 mW, and the integration time was 10,000 ms. Then, 20 μL HCR products were mixed with 20 μL of 2.86 mM DAPI (Raman signal molecule)

and reacted in the dark for 10 min. Subsequently, 60 μL AgNP colloid was added and reacted in dark for 5 min. Finally, 10 μL of this liquid sample was taken on a glass plate and detected after dark subtracted.

2.7 Performances of this dual signal amplification system

Three non-target bacteria samples, *E. coli* being a representative of Gram-negative bacteria, *S. aureus* being a representative of Gram-positive bacteria, and *M. smegmatis* being a representative of actinomycetes, were used to test the specificity of this method for the detection of target *S. typhimurium*, as these three are common bacteria of different species in the surrounding environment that may also contaminate drinking water or food. The concentration of these non-target bacteria was at least 10^6 CFU/mL while that of S. typhimurium was at a lower concentration of 10^4 CFU/mL. The detection procedure was the same as that for *S. typhimurium*.

To verify the effectiveness of this method in detecting real samples, commercially available UHT milk was used for spike experiment. 10 mL milk sample was centrifuged at 8000 rpm for 8 min to remove the upper layer of cream. Subsequently, serial unknown concentrations of *S. typhimurium* were added into these samples, and then detected simultaneously both by this proposed method and the traditional plate counting method. The results were compared. For solid foods, on the other hand, as long as they can be mashed or washed into sterile water, their detection steps are the same as the above-mentioned liquid foods.

3. Results and discussion

3.1 Principle of the detection

The schematic illustration of the principle of this aptasensor based immuno-HCR-SERS method with dual signal amplification system for detecting *S. typhimurium* was shown in Fig. 1. IMBs could specifically capture *S. typhimurium* via antigen-antibody immuno reaction. When the hairpin-structural aptamer modified with initiator binds to the targets, the hairpin structure would be opened and exposed the triggering sequence. Subsequently, H1 and H2 alternately opened the stem-loop structure and complemented each other, eventually extending a nicked dsDNA. Then excess DAPIs were added as Raman signal molecule, which can be deeply embedded in the AT region of the dsDNA. The DAPIs embedded in the dsDNA hardly display SERS signal while the free DAPIs show intense SERS signal at about 1610 cm^{-1} due to the C=N stretching mode when adsorbed on the surface of AgNPs [29]. Therefore, the more target bacteria were there, the more dsDNA extended while less free DAPIs remained thus abtained weaker SERS signal. That meant the SERS signal was negatively correlated with the bacterial concentration and was therefore used to determine the amount of *S. typhimurium*.

3.2 Characterization of the AgNPs and IMBs

The morphology and particle size of the Raman substrate play an important role in ensuring the formation of hot spots and normal Raman scattering [30, 31]. Fig. S1 showed the UV-Vis absorption spectroscopy and particle size distribution of the prepared AgNPs. As shown in Fig. S1A, there were a main peak at 485nm and a small peak at 350 nm, indicated good morphology uniformity of the AgNPs. The particle size of AgNPs was mainly distributed around 65 nm, with only a very low ratio below 10 nm and above 200 nm (Fig. S1B). Fig. S2 showed the UV-Vis absorption spectroscopy of the prepared IMBs. The experimental group 1 and 2 showed significant characteristic absorption peaks at 450 nm while the control group didn't, which indicated the anti-*S. typhimurium* antibody had been successfully coupled to the surface of carboxyl magnetic beads.

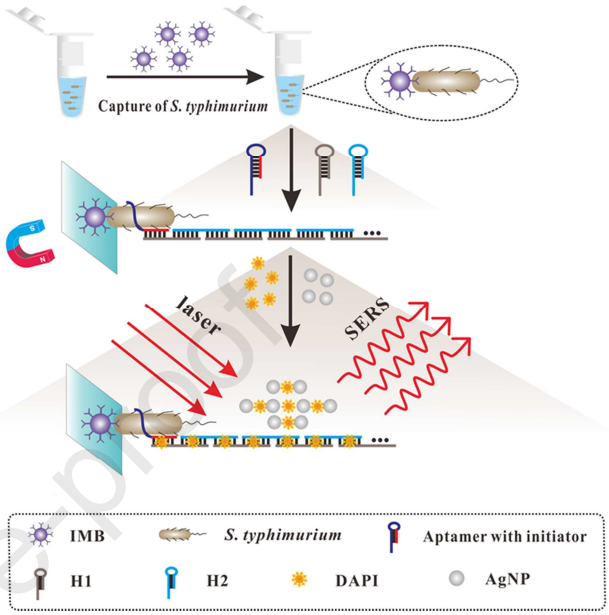

Figure 1. Principle of the aptasensor based Immuno-HCR-SERS method for the detection of *S. typhimurium*.

3.3 Determination of optimal conditions for HCR

The results of the combinations of different experimental conditions were shown in Fig. S3, these diffuse bands were the extended dsDNA of different length. The length of the extended dsDNA gradually increased as the reaction time increased at room temperature. The result was the opposite while the temperature was at 42 °C. The best extensions were performed at 37 °C. In order to achieve the best extension efficiency in the shortest time, reacting at 37 ° C for 1 h had been chosen as the optimized conditions for HCR.

3.4 Detection results for *S. typhimurium*

As shown in Fig. 2A, the strongest Raman peak appeared at 1610 cm^{-1} in each gradient group and the peak intensity decreases with the concentration of target bacteria increases. Thus, the peak values at 1610 cm^{-1} were used to quantitatively assess the concentration of *S. typhimurium*. The linear fitting analysed by Origin 2017 was shown in Fig. 2B. A good linear relationship was achieved between the SERS intensities and the logarithms of *S. typhimurium* concentrations in the range from 10 to 10^5 CFU/mL and the theoretical LOD of 6 CFU/mL. Comparison of the proposed dual signal amplification Immuno-HCR-SERS method and recently reported methods are shown in Table 1. All these methods have made great progress in increasing sensitivity or shortening the detection time, while our method has also almost reached the level of single molecule detection.

3.5 Specificity of the assay by using Immuno-HCR-SERS method

Compared with the control (no bacteria were added) and three other non-target bacteria, the SERS intensity of *S. typhimurium* was much lower than those of the control and the non-target bacteria (shown in Fig. 3). This was due to the good affinity between the selected aptamer and the target bacteria, suggesting the good specificity and selectivity of the Immuno-HCR-SERS method for the detection of *S. typhimurium* (10^4 CFU/mL) and three other different non-target bacteria *E. coli*, *M. smegmatis* and S. aureus (10^6 CFU/mL). In addition, the strain difference is the only variable in these experiments, which means that the changes in SERS intensities are caused by this factor rather than others.

Table 1. Comparison of this proposed method and the recently reported methods for the detection of *S. typhimurium.*

Method	LOD (CFU/mL)	Linear range (CFU/mL)	Assay time	Reference
SERS-based aptasensor	15	15-1.5×10^6	-	[32]
Label-free impedimetric biosensor	3	10^2 - 10^8	~ 45 min	[33]
PCR method with immunoseparation	100	10^2 - 10^5	-	[34]
Aptamer based SERS detection	35	10^2 - 10^7	~ 1 h	[24]
SERS aptasensor	4	10^1 - 10^5	-	[35]
Aptamer-based F0F1-ATPase biosensor	10	10 ^{1}- 10^4	-	[36]
Capillary biosensor	14	300 – 3.0×10^6	-	[37]
Colorimetric detection	16	10^2 - 10^9	-	[38]
Aptasensor based immuno-HCR-SERS	6	10^1 - 10^5	~ 3.5 h	This work

Table 2. Comparison of the amount of *S. typhimurium* measured by both plate counting method and this proposed method (n=3).

UHT milk sample	Amounts counted by plate counting method (CFU/mL)	Amounts detected by this proposed method (CFU/mL)	Recovery rate (%)
1	5307 ± 133	5534 ± 253	104.27
2	4367 ± 208	4532 ± 241	103.77
3	231 ± 6	238 ± 42	103.08
4	41 ± 3	43 ± 8	104.88

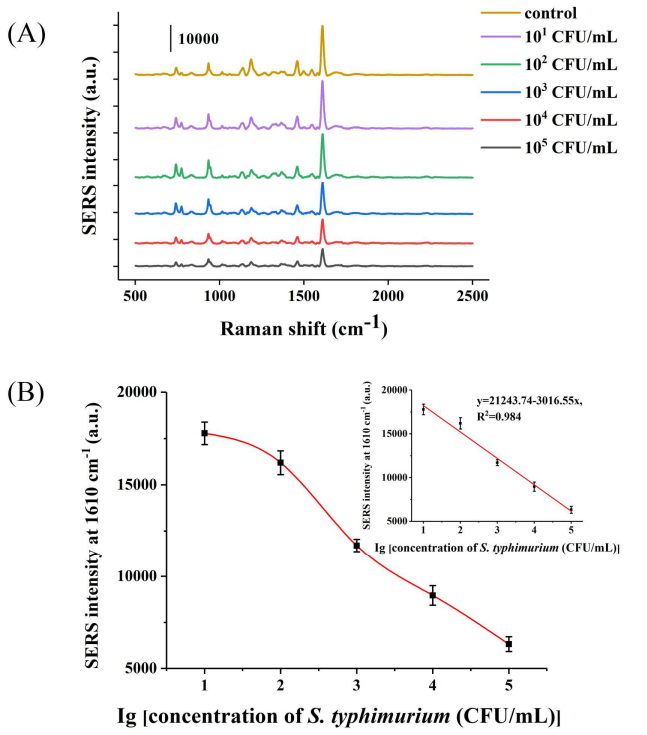

Figure 2. (A) Raman scattering spectra of *S. typhimurium* with a series of concentration gradient (top to bottom, 10^1 CFU/mL, 10^2 CFU/mL,10^3 CFU/mL, 10^4 CFU/mL and 10^5 CFU/mL). (B) Calibration curve by plotting the SERS intensities at 1610 cm^{-1} against logarithms of the concentrations of *S. typhimurium*. The error bars represent the deviations of three parallel experiments.

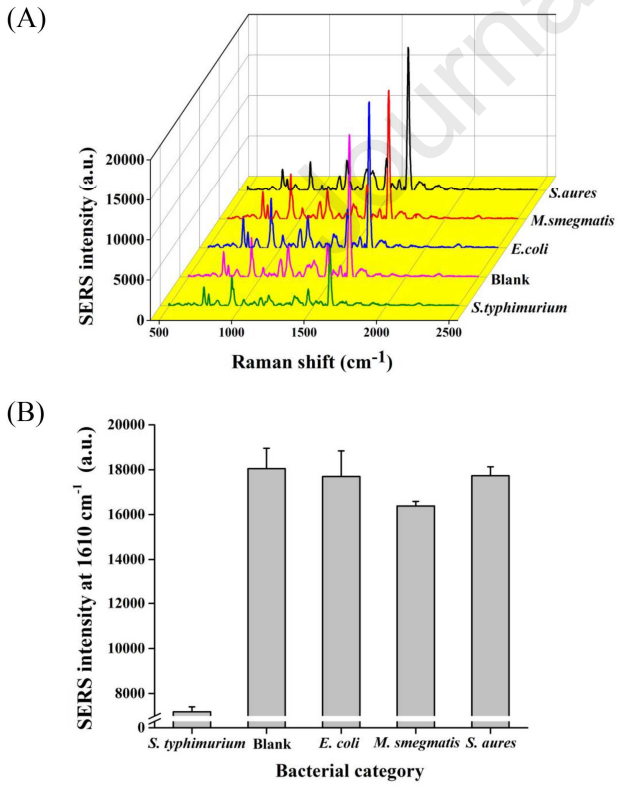

Figure 3. (A) Raman scattering spectra and (B) SERS intensities at 1610 cm^{-1} of *S. typhimurium*, blank and three other common bacteria.

3.6 Sample detection by aptasensor based immuno-HCR-SERS method

The results of the tests for spiked milk sample using the Immuno-HCR-SERS method were shown in Table 2, with the conventional plate count method carried out parallelly as the measuring standard refer. The results showed a good consistency between the two methods. The recovery rates determined by dividing the results of the proposed method by those of the traditional standard method were between 103.08% to 104.88%. This might be due to the fact that *S. typhimurium* spiked into milk samples were not strictly during the exponential phase, so IMBs could also capture the injured or dead ones that did not grow on the plate. It was undeniable that these bacteria still preserve pathogenicity due to the expression of virulence genes [39-41].

4. Conclusion

In this work, we presented an aptasensor based immuno-HCR-SERS method with dual signal amplification capability for highly sensitive detection of *S. typhimurium*. The proposed method fully exploits the advantages of nucleic acid aptamer to specifically recognize and attach to the target, HCR triggered to amplify the signal, and finally SERS utilized to convert and further amplification of the signal. It thereby realizing the sensitive and rapid detection of *S. typhimurium* with the LOD of 6 CFU/mL and the detection time of 3.5 h. Moreover, this method exhibited good specificity and could be well applied to the detection of real samples, all of these make it promising to prevent the occurrence of foodborne diseases thus ensuring food safety in the future.

Acknowledgements

This study was supported by the National Natural Science Foundation of China 31571929 and 31730004, the Fundamental Research Funds for the Central Universities and Open Funding Project of State Key Laboratory of Dairy Biotechnology (SKLDB).

CRediT authorship contribution statement

Ao Li: Investigation, Experiments, Formal analysis, Software, Writing – original draft. **Peng Zuo:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Bang-Ce Ye:** Resources, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

There are no conflicts to declare.

References

[1] A.M. Melo, D.L. Alexandre, R.F. Furtado, M.F. Borges, E.A. Figueiredo, A. Biswas, H.N. Cheng, C.R. Alves, Electrochemical immunosensors for Salmonella detection in food, Appl. Microbiol. Biot. 100 (2016) 5301-5312.

[2] R. Brandt, A. Petersen, S. Brix, T.R. Licht, H. Frøkiær, Epithelial entry rather than the ensuing systemic immune response determines the pathogenicity of two Salmonella enterica serovar typhimurium strains in a mouse model, Microbes Infect. 15 (2013) 911-919.

[3] S.E. Majowicz, J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien, T.F. Jones, A. Fazil, R.M. Hoekstra, The global burden of nontyphoidal Salmonella gastroenteritis, Clin. Infect. Dis. 50 (2010) 882-889.

[4] M.H. Kothary, U.S. Babu, Infective dose of foodborne pathogens in volunteers: a review, J. Food Safety 21 (2010) 49-68.

[5] C. Techathuvanan, D.H. D'Souza, Reverse-transcriptase loop-mediated isothermal amplification as a rapid screening / monitoring tool for Salmonella enterica detection in liquid whole eggs, J. Food Sci. 77 (2012) M200-M205.

[6] W. Wang, L. Liu, S. Song, L. Tang, H. Kuang, C. Xu, A highly sensitive ELISA and immunochromatographic strip for the detection of Salmonella typhimurium in milk samples, Sensors 15 (2015) 5281-5292.

[7] S.H. Flint, N.J. Hartley, Evaluation of the TECRA immunocapture ELISA for the detection of Salmonella typhimurium in foods, Lett. Appl. Microbiol. 17 (2010) 4-6.

[8] J. Alves, E.Y. Hirooka, Development of a multiplex real-time PCR assay with an internal amplification control for the detection of Campylobacter spp. and Salmonella spp. in chicken meat, LWT - Food Sci. Technol. 72 (2016) 175-181.

[9] L. Wang, R. Wang, H. Wang, M. Slavik, H. Wei, Y. Li, An aptamer-based PCR method coupled with magnetic immunoseparation for sensitive detection of Salmonella typhimurium in ground turkey, Anal. Biochem. 533 (2017) 34-40.

[10] P.P. Kumar, R.K. Agarwal, P. Thomas, B. Sailo, A. Prasannavadhana, AshokKumar, J.L. Kataria, D.K. Singh, Rapid detection of Salmonella enterica subspecies enterica serovar typhimurium by loop mediated isothermal amplification (LAMP) test from field chicken meat samples, Food Biotechnol. 28 (2014) 50-62.

[11] C. Pestourie, B. Tavitian, F. Duconge, Aptamers against extracellular targets for in vivo applications, Biochimie 87 (2005) 921-930.

[12] C.A. Savran, S.M. Knudsen, A.D. Ellington, S.R. Manalis, Micromechanical detection of proteins using aptamer-based receptor molecules, Anal. Chem. 76 (2004) 3194-3198.

[13] K. Takemura, P. Wang, I. Vorberg, W. Surewicz, S.A. Priola, A. Kanthasamy, R. Pottathil, S.G. Chen, S. Sreevatsan, DNA aptamers that bind to PrP(C) and not PrP(Sc) show sequence and structure specificity, Exp. Biol. Med. 231 (2006) 204-214.

[14] N. Duan, S. Wu, C. Zhu, X. Ma, Z. Wang, Y. Yu, Y. Jiang, Dual-color upconversion fluorescence and aptamer-functionalized magnetic nanoparticles-based bioassay for the simultaneous detection of Salmonella typhimurium and Staphylococcus aureus, Anal. Chim. Acta 723 (2012) 1-6.

[15] N. Ansari, R. Yazdian-Robati, M. Shahdordizadeh, Z. Wang, K. Ghazvini, Aptasensors for quantitative detection of Salmonella typhimurium, Anal. Biochem. 533 (2017) 18-25.

[16] P. Liu, X. Yang, S. Sun, Q. Wang, K. Wang, J. Huang, J. Liu, L. He, Enzyme-free colorimetric detection of DNA by using gold nanoparticles and hybridization chain reaction amplification, Anal. Chem. 85 (2013) 7689-7695.

[17] Z. Wu, G.Q. Liu, X.L. Yang, J.H. Jiang, Electrostatic nucleic acid nanoassembly enables hybridization chain reaction in living cells for ultrasensitive mRNA imaging, J. Am. Chem. Soc. 137 (2015) 6829-6836.

[18] L. Zhang, Y. Liu, L. Ying, Y. Zhao, W. Wei, S. Liu, Sensitive electrochemical assaying of DNA methyltransferase activity based on mimic-hybridization chain reaction amplified strategy, Anal. Chim. Acta 933 (2016) 75-81.

[19] L. Liang, F. Lan, L. Li, S. Ge, J. Yu, R. Na, H. Liu, Y. Mei, Paper analytical devices for dynamic evaluation of cell surface N-glycan expression via a bimodal biosensor based on multibranched hybridization chain reaction amplification, Biosens. Bioelectron. 86 (2016) 756-763.

[20] W. Zhou, B.C. Yin, B.C. Ye, Highly sensitive surface-enhanced Raman scattering detection of hexavalent chromium based on hollow sea urchin-like TiO2@Ag nanoparticle substrate, Biosens. Bioelectron. 87 (2017) 187-194.

[21] Y.C. Ou, J.A. Webb, C.M. O'Brien, I.J. Pence, E.C. Lin, E.P. Paul, D. Cole, S.H. Ou, M. Lapierre-Landry, R.C. Delapp, Diagnosis of immunomarkers in vivo via multiplexed surface enhanced Raman spectroscopy with gold nanostars, Nanoscale 10 (2018) 13092-13105.

[22] J. Cheng, S. Zhang, S. Wang, P. Wang, X.O. Su, J. Xie, Rapid and sensitive detection of acrylamide in fried food using dispersive solid-phase extraction combined with surface-enhanced Raman spectroscopy, Food Chem. 276 (2019) 157-163.

[23] N. Duan, B. Chang, H. Zhang, Z. Wang, S. Wu, Salmonella typhimurium detection using a surface-enhanced Raman scattering-based aptasensor, Int. J. Food Microbiol. 218 (2016) 38-43.

[24] X. Xu, X. Ma, H. Wang, Z. Wang, Aptamer based SERS detection of Salmonella typhimurium using DNA-assembled gold nanodimers, Microchim. Acta 185 (2018) 325.

[25] X. Ma, X. Xu, Y. Xia, Z. Wang, SERS aptasensor for Salmonella typhimurium detection based on spiny gold nanoparticles, Food Control 84 (2017) 232-237.

[26] R. Joshi, H. Janagama, H.P. Dwivedi, T.M. Senthil Kumar, L.A. Jaykus, J. Schefers, S. Sreevatsan, Selection, characterization, and application of DNA aptamers for the capture and detection of Salmonella enterica serovars, Mol. cell. Probe. 23 (2009) 20-28.

[27] R.M. Dirks, N.A. Pierce, Triggered amplification by hybridization chain reaction, P. Natl. Acad. Sci. USA. 101 (2004) 15275-15278.

[28] P.C. Lee, D. Meisel, Adsorption and surface-enhanced Raman of dyes on silver and gold sols, J. Phys. Chem. 86 (1982) 3391-3395.

[29] X. Dou, T. Takama, Y. Yamaguchi, K. Hirai, H. Yamamoto, S. Doi, Y. Ozaki, Quantitative analysis of double-stranded DNA amplified by a polymerase chain reaction employing surface-enhanced Raman spectroscopy, Appl. Optics 37 (1998) 759-763.

[30] M.A. Shenashen, S.A. El-Safty, E.A. Elshehy, Synthesis, morphological control, and properties of silver nanoparticles in potential applications, Part. Part. Syst. Char. 31 (2014) 293-316.

[31] Y. Zhao, Y.J. Zhang, J.H. Meng, S. Chen, R. Panneerselvam, C. Y. Li, S.B. Jamali, X. Li, Z.L. Yang, J.F. Li, Z.Q. Tian, A facile method for the synthesis of large-size Ag nanoparticles as efficient SERS substrates, J. Raman Spectrosc. 47 (2016) 662-667.

[32] N. Duan, B. Chang, Z. Hui, Z. Wang, S. Wu, Salmonella typhimurium detection using a surface-enhanced Raman scattering-based aptasensor, Int. J. Food Microbiol. 218 (2016) 38-43.

[33] E. Sheikhzadeh, M. Chamsaz, A.P. Turner, E.W. Jager, V. Beni, Label-free impedimetric biosensor for Salmonella typhimurium detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer, Biosens. Bioelectron. 80 (2016) 194-200.

[34] L. Wang, R. Wang, W. Hong, M. Slavik, W. Hua, Y. Li, An

aptamer-based PCR method coupled with magnetic immunoseparation for sensitive detection of Salmonella typhimurium in ground turkey, Anal. Biochem. 533 (2017) 34-40.

[35] X. Ma, X. Xu, Y. Xia, Z. Wang, SERS aptasensor for Salmonella typhimurium detection based on spiny gold nanoparticles, Food Control 84 (2018) 232-237.

[36] N. Duan, W. Sun, S. Wu, L. Liu, X. Hun, Z. Wang, Aptamer-based F0F1-ATPase biosensor for Salmonella typhimurium detection, Sensor. Actuat. B-Chem. 255 (2018) 2582-2588.

[37] H. Zhang, L. Xue, F. Huang, S. Wang, L. Wang, N. Liu, J. Lin, A capillary biosensor for rapid detection of Salmonella using Fe-nanocluster amplification and smart phone imaging, Biosens. Bioelectron. 127 (2019) 142-149.

[38] J. Yi, P. Wu, G. Li, W. Xiao, L. Li, Y. He, Y. He, P. Ding, C. Chen, A composite prepared from carboxymethyl chitosan and aptamer-modified gold nanoparticles for the colorimetric determination of Salmonella typhimurium, Microchim. Acta 186 (2019) 1-8.

[39] G.S. Chilcott, K.T. Hughes, Coupling of flagellar gene expression to flagellar assembly in Salmonella enterica serovar typhimurium and Escherichia coli, Microbiol. Mol. Biol. Rev. 64 (2000) 694-708.

[40] M. Mcclelland, K.E. Sanderson, J. Spieth, S.W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, Complete genome sequence of Salmonella enterica serovar Typhimurium LT2, Nature 413 (2001) 852-856.

[41] D.A.C. Stapels, P.W.S. Hill, A.J. Westermann, R.A. Fisher, T.L. Thurston, A.E. Saliba, I. Blommestein, J. Vogel, S. Helaine, Salmonella persisters undermine host immune defenses during antibiotic treatment, Science 362 (2018) 1156-1160.

Highlights

- A signal-cascade-amplification method named as “immuno-HCR-SERS” was constructed for the detection of *S. typhimurium*.
- *S. typhimurium* aptamer modified with a trigger sequence to both specifically recognize targets and trigger HCR.
- Immuno-HCR followed by SERS for the further signal amplification to realize the highly sensitive detection of *S. typhimurium.*