Microchim Acta (2016) 183:741–748
DOI 10.1007/s00604-015-1715-1

ORIGINAL PAPER

Lysozyme as sensitive reporter for fluorometric and PCR based detection of *E. coli* and *S. aureus* using magnetic microbeads

Yan Zeng[1,2] · Yi Wan[1] · Dun Zhang[1]

Received: 23 September 2015 / Accepted: 3 December 2015 / Published online: 21 December 2015
© Springer-Verlag Wien 2015

Abstract We describe the use of lysozyme as a sensitive reporter for detection of bacteria. The assay comprises the following steps: First, specific primers are used for targeting the rDNA of E. coli and S. aureus, respectively, and the polymerase chain reaction is applied to amplify bacterial DNA. Then, the target DNA binds to the capture probe immobilized on magnetic beads, with lysozyme acting as signal reporter that catalyzes the hydrolysis of an enzyme substrate to form the blue fluorescent product 4-methylumbelliferone. Coupled with multiple lysozyme substrates, this fluorometric detecting method has a detection limit of 50 CFU·mL^{-1} bacteria. The optical changes may also be detected by colorimetry and monitored with bare eyes. The method was selective over other bacteria and was successfully applied to the determination of E. coli and S. aureus in human serum, which provides a perspective for lysozyme application and species-specific pathogenic bacteria detection.

Keywords Bacterial detection · Fluorescence · Colorimetry · Bare eye detection · Polymerase chain reaction · Magnetic beads

Electronic supplementary material The online version of this article (doi:10.1007/s00604-015-1715-1) contains supplementary material, which is available to authorized users.

✉ Dun Zhang
zhangdun@qdio.ac.cn

[1] Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China

[2] University of the Chinese Academy of Science, 19 (Jia) Yuquan Road, Beijing 100039, China

Introduction

Much effort has been devoted to the study of methods for species-specific pathogenic bacteria detection which plays an important role in food poisoning, disease spreading and environmental monitoring [1, 2]. In past decade, bacteria culture-based assay remains clinical gold standard, even though it is time-consuming. Other immunological approaches using surface biological and chemical recognition-site as recognizer are afflicted by false positive reaction and low sensitivity [3]. Polymerase chain reaction (PCR) has been widely used for the detection of bacterial DNA, not only because it is far more rapid than traditional culturing, it can also amplify specific regions of bacterial genome exponentially within couple of hours, which makes it more accurate and sensitive for species-specific bacteria detection [4]. In this way, species-specific bacteria can be detected with PCR-amplified bacterial DNA as targets, as any self-replicating organism can be discriminated from another on the basis of nucleic acid sequences unique to that particular organism [5].

Fluorometric sequence-specific DNA detection is attractive because it is easily performed and rapid, and therefore is widely used [6]. In general, DNA molecules are used as the recognition moiety, as single-stranded DNA (ssDNA) can hybridize to complementary nucleic acids with the high specificity of Watson-Crick base paring. To convert this hybridization event into fluorescent response for instrument output, many signaling mechanisms have been reported to achieve effective transduction, including (1) labeling oligonucleotides with fluorescent dyes or fluorescent polymers; (2) immobilizing them onto fluorescent microspheres or quantum dots surface; (3) conjugating them with biological components which will cause a change in fluorescence [7]. Among the biological components, enzyme is the most appropriate elements because it has high

chemical specificity via various enzymatic reactions and sensitive detection with inherent catalytic signal amplification [8–10]. In this case, enzyme-DNA conjugate is employed as biocatalyst to achieve fluorescence transduction of nucleic acid recognition, considering it has unique properties of DNA and unlimited variety of enzyme functionality [11, 12]. So which kind of enzyme can be used to establish the link?

Lysozyme (1, 4-N-acetylmuramidase) can provide this link. Since the initial discovery of lysozyme in tears and nasal secretions by Alexander Fleming in 1922, it has become one of the most thoroughly characterized enzymes [13]. Huge literature has accumulated on its structure, function, genetics, activity, and properties [14]. However, few approaches based on its catalytic ability and signal amplification advantages have been published, and even fewer have been explored for Species-specific pathogenic bacteria detection. With catalytically active site of Asp52 and Glu35, lysozyme can hydrolyze the substrate 4-methylumbelliferyl-β-D-N, N′, N″-triacetylchitotrioside and produce a fluorescent product 4-methylumbelliferone which can be detected with fluorescence spectroscopy [15]. In this way, a direct relationship between the concentrations of lysozyme and fluorescence signal can therefore be established. More importantly, the released methylumbelliferone can even be reliably measured at concentrations of 0.1 nM under ambient conditions, which was obviously attributed to the highly efficient enzymatic turnover of lysozyme [16]. The above properties of lysozyme make it an ideal catalyst for bacteria detection as sensitive reporter.

Here, we provided lysozyme as sensitive reporter, and tested its application in bacteria detection. By involving specific primers that target bacterial DNA with polymerase chain reaction, the amplified bacterial DNA was obtained. Then, the system is based on target bacterial DNA binding with capture probe immobilized on magnetic beads and lysozyme acted as signal reporter, which can catalyze enzyme substrate into optical signal.

Experimental

Materials

Lysozyme, 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (sulfo-SMCC), 4-methylumbelliferyl-β-D-N,N′,N′′-triacetylchitotrioside, dithiothreitol (DTT), 4-nitrophenyl-β-D-N, N′,N″-triacetylchitotriose were ordered from Sigma-Aldrich (Shanghai, China, http://www.sigmaaldrich.com). Streptavidin-modified magnetic beads (MBs 1.0 μm, 10 mg.mL^{-1}) and Amicon centrifugal filters (3 kDa molecular weight cutoff) was purchased from Life Technologies AS (Shanghai, http://www.thermofisher.com) and Millipore Inc. (Billerica, MA, http://www.emdmillipore.com), respectively. Taq DNA polymerase, 6 × DNA PAGE loading buffer, protein and DNA marker was purchased from Takara Biotechnology Co., Ltd. (Dalian, China, http://www.takara.com.cn). Human serum was obtained from Haici Hospital (Qingdao, China, http://www.qdhaici.cn), All Olignoculeotides and primers were custom-synthesized and HPLC purified by Sangon Biotechnology Co., Ltd. (Shanghai, China, http://www.sangon.com), and the sequences were shown in Table S1. All chemicals and solutions used throughout this study were dissolved or diluted with ultrapure water.

Bacteria culture

All bacteria were offered from the Key Laboratory of Experimental Marine Biology. Bacteria were seeded and cultured in suspension using Luria-Bertani media. For the bacteria, a single colony was inoculated in bacterial medium at 30 °C overnight. After centrifugation (5437.5 g, 10 min) and PBS washing, the bacterial cells were diluted to the desired concentration in phosphate buffer. Bacterial cell numbers were estimated by plating onto standard agar plates.

DNA extraction

For DNA extraction, bacteria with different concentrations were treated by Tianamp Bacteria DNA kit. E. Coli was selected as model bacteria throughout the whole process of experiment, and the target amplicon and primer set were designed as previously used [1]. The extracted DNA was amplified using Taq DNA polymerase and specific primers for bacteria, and the reagents amount were added as follows: Template DNA, 5 μL; ultrapure water, 15.8 μL; polymerase buffer, 2.5 μL; dNTPs, 0.5 μL; forward primer, 0.5 μL; reverse primer, 0.5 μL; taq polymerase, 0.2 μL. The following thermal cycling conditions were used: initiation (94 °C, 5 min); 35 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), extension (94 °C, 30 s); and termination (72 °C, 7 min). The final PCR products were validated by polyacrylamide gel electrophoresis (PAGE).

Magnetic microbead (MB)-DNA conjugations

During the experiment, 1 mL streptavidin-modified MBs was washed five times with washing buffer (10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, pH 7.5), and the ultimate magnetic absorptions were resuspended in 1 mL binding buffer (10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, pH 7.5). Then, the streptavidin-modified MB suspension were mixed with 1 mL of 5 μM biotin-modified capture probes, and the mixture was incubated at 25 °C for 30 min with gentle shaking. After

repeated magnetic separation and cleaning, the weekly bound capture probes would be removed, and the rest absorption was dispersed in 1 mL reaction buffer (20 mM Tris-HCl, 5 mM $MgCl_2$, pH 8.0) and stored at 4 °C for further use.

Lysozyme-DNA conjugation

For lysozyme-DNA conjugation, 200 μL of 10 mg·mL^{-1} lysozyme in buffer A (0.1 M NaCl, 0.1 M sodium phosphate buffer) was mixed with 0.25 mg of sulfo-SMCC. After vortexing for 5 min, the solution was placed on a shaker for 1 h at room temperature. Then, the mixture was centrifuged to remove the insoluble sulfo-SMCC, while the supernatant was purified for 8 times by Amicon-3 k using buffer A. For DNA activation, Thiol-modified oligonucleotide probes (10^{-4} M, 200 μL) were treated with 125 mM dithiothreitol in PBS with 10 mM sodium hydroxide for 2 h and then purified by Amicon-3 K using buffer A for 8 times. The above solution of DNA-SH was then mixed with sulfo-SMCC activated lysozyme, and the mixed solution was kept at room temperature for 48 h. To remove unconjugated DNAs and lysozyme, the solution was purified by Amicon-3 K using buffer A for 8 times.

Procedures for target detection

The detecting system of bacteria based on lysozyme-DNA as reporter was performed by mixing 10 μL MB-capture probe, 10 μL lysozyme-DNA probe, and different concentrations of target bacteria DNA to a final volume of 30 μL, followed by incubating at 37 °C for 30 min. After that, the MBs were separated from the solution by magnetic rack, washed 3 times with washing buffer, and then dispersed in 100 μL PBS. The resulting solution was then treated with lysozyme substrate for signal collection. Each experiment was repeated at least three times to test reproducibility.

Signal detection with fluorometric assay

For fluorescence signal detection, 100 μL of the above resulting solution was mixed with 100 μL of lysozyme fluorescence Substrate (1.5 g·L^{-1}). The mixed solution was kept at 50 °C for 1.5 h with reaction buffer (20 mM PBS pH 5.0). At the end of the incubation period, the reaction mixture was taken and terminated by addition of 200 μL bicarbonate buffer (pH 10.4) [17]. The resulting fluorescent product was measured at 455 nm (λ_{ex} = 360 nm).

Signal detection by colorimetry

For colorimetry detection, experiment on enzymatic hydrolysis was performed in sodium citrate buffer (pH 5.0), containing 100 μL of the above resulting solution, 100 μL of lysozyme colorimetric substrate (1.2 mM). The mixed solution was kept at 40 °C for 1.5 h. At the end of the incubation period, the reaction mixture was taken and terminated by addition of 200 μL borate buffer (pH 9.3) [18]. The chromophore was measured at 400 nm.

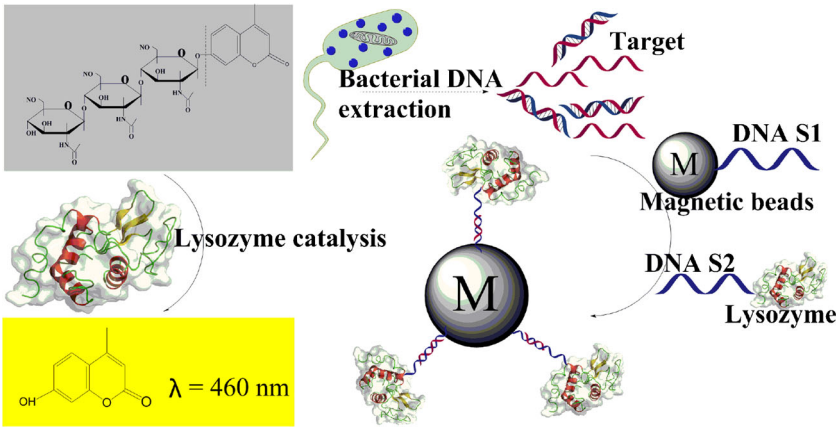
Results and discussion

Detection principle

Scheme 1 illustrates the principle of bacteria detection with lysozyme as reporter. To select a target region for amplification and hybridization, we used *E. coli* as model bacteria. The specific primer sequences were obtained from Chung's work, and all the oligonucleotide sequences of capture probe (S1) and signal probe (S2) conjugated with lysozyme were designed complementary to the bacteria amplicon sequence [1]. Firstly, using specific primers for *E. coli*, target regions were amplified by PCR to produce large numbers of single-strand DNA including specific sequences. Then, the produced DNAs were captured by S1 immobilized on MBs, and the overhanging edges of target DNA would hybridize with S2. In this way, a sandwich DNA hybridization including MB-capture probe, target DNA and reporter probe was formed. Taking advantage of catalytic ability of lysozyme, fluorescence signal would be captured.

Magnetic microbeads were selected as carrier materials in this study. In most approaches, the use of MBs facilitates an easy separation which mainly reflects the high efficiency and specificity of binding between capture probe and target [19, 20]. It plays another important role in purifying lysozyme-DNA conjugates [21]. Conjugating approaches with noncovalent or covalent interaction for DNA-protein conjugates have been well developed. However, most approaches are not effective enough which makes large amounts of unconjugated DNAs and protein molecules keep in a Free State even after cycles of chromatography [11, 12]. By employing MBs as carrier, it allows not only sample capture, but also makes it more simple and efficient to achieve purification of the conjugates by washing procedures. On the other hand, in the absence of target DNA which acts the role of "bridge", MBs would not combine with lysozyme, and no fluorescence readout would be obtained.

As the amino acid side-chains glutamate 35 (Glu35) and aspartate 52 (Asp52) are critical to the activity of this enzyme, the reactive amines that are not involved in the active site are chosen as the sites for conjugation. This preserves the catalytic activity of lysozyme best. As shown in Fig. 1a, for lysozyme conjugation, lysozyme was first functionalized through its surface amines by a commercial cross-linkers sulfo-SMCC, and then it would conjugate with DTT activated thiol-modified DNA oligonucleotides via the maleimide-thiol



Scheme 1 Fluorometric and PCR sensing mechanism of E. coli and S. aureus detection with lysozyme as sensitive reporter and magnetic microbeads as carrier

reaction to yield lysozyme-DNA conjugates [19]. During each step, ultra-filtration separation was carried out to remove excess unconjugated DNA. With synthesized bacterial DNA segment as target DNA, we evaluated for the binding ability of lysozyme-DNA conjugates and specificity against target DNA. As shown from Fig. 1b, no fluorescent signal is observed without the addition of lysozyme-DNA conjugates. Higher-intensity fluorescent signal was observed from the target DNA than from the control DNA and blank DNA, which indicated that the lysozyme-DNA maintained not only the enzyme activity but also the DNA-binding ability and specificity.

Assay performance

To demonstrate the methodology shown in Scheme 1, we investigated the detection of *E. coli* with fluorescence spectrometry. PCR was carried out using the genomic DNA extracted from each concentration of *E. coli* cells. Then, the PCR products were characterized by PAGE which was shown in Fig. 2a. All bands were shown between 60 bp and 80 bp, which were completely consistent with theoretical length of DNA amplicons. With the decrease of bacteria concentrations, the electrophoresis band is getting weak with the most light band of 5×10^3 CFU·mL^{-1} bacteria amplified DNA. This phenomenon can be attributed to the limited GelRed staining for ssDNA. The fluorometirc system was then applied to analyze DNAs extracted from bacteria. As shown in Fig. 2b, the absence of bacteria DNA produced negligible fluorescent signal because the absence of DNA facilitated sandwich DNA hybridization.

The introduction of MBs also leads to separation of unconjugated lysozyme. Hence, background signal is strongly reduced. In the presence of 5×10^8 CFU·mL^{-1} bacteria, more than 15-fold enhancement of fluorescent intensity was observed in comparison with that in the absence of target DNA. The fluorescent intensity is linear to the logarithm of bacteria concentration ranging from 5×10^1 to 5×10^8 CFU·mL^{-1}, with a detection limit of 50 CFU·mL^{-1} *E. coli* (Fig. 2), which was comparable with other methods listed in Table S2. The linear regression equation is Y = 99.655 lgx-116.48, with a correlation coefficient of 0.9888. All these dates were collected under optimal experimental conditions (shown in Figure S1).

As mentioned above, the fluorescence signal was produced via the enzymatic hydrolysis of substrate by lysozyme-DNA conjugate combined with MBs. So the fluorescence signal should be dependent on the concentration of lysozyme

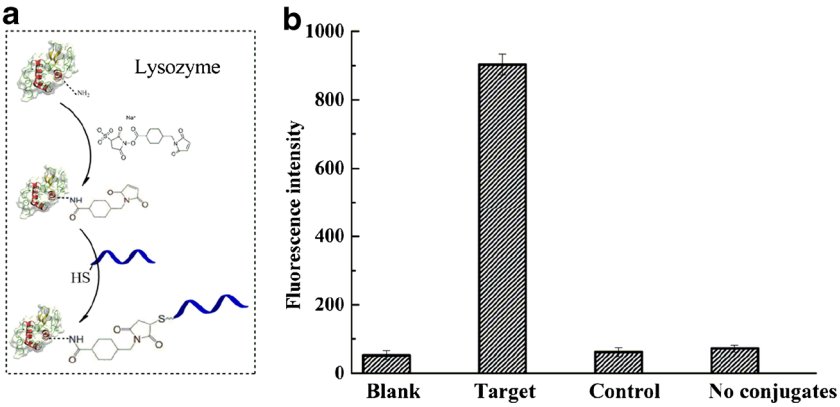


Fig. 1 **a** Assembly of signal probe **b** Binding ability and specificity of Lysozyme-DNA

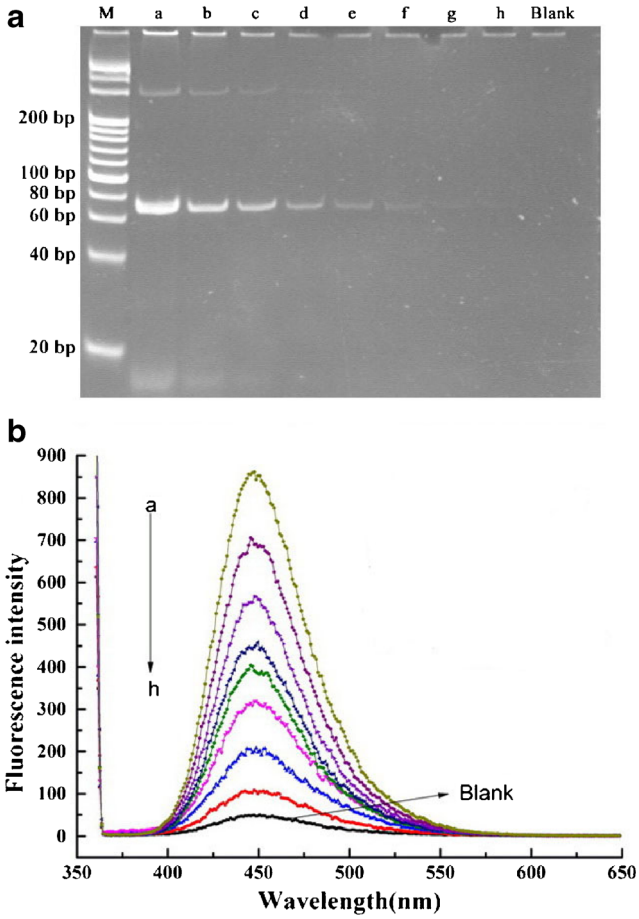


Fig. 2 **a** PAGE photos of PCR products of Sample (a) 5×10^8, (b): 5×10^7, (c): 5×10^6, (d): 5×10^5, (e): 5×10^4, (f): 5×10^3, (g): 5×10^2, (h): 5×10^1 CFU·mL^{-1} E. coli and Blank, **b** Fluorescence spectrum to PCR products obtained from serial dilutions of E.coli in the range of 5×10^1–5×10^8 CFU·mL^{-1}

immobilized on MBs or the concentration of target DNA in the sample with excess amount of lysozyme substrate. However, as the diameter of MB is fixed, the capture probe coverage on one MB would reach saturation [22]. As a result, the target DNA and lysozyme-DNA conjugates immobilized on one MB would also reach saturation. To demonstrate this inference, bacteria samples with concentration higher than 5×10^8 CFU·mL^{-1} was detected. As shown in Figure S2, there is little difference of Fluorescence intensity between sample A (5×10^8 CFU·mL^{-1} bacteria) and sample B, C, D, which indicated that this system cannot applied to the detection of bacteria in concentrations higher than 5×10^8 CFU·mL^{-1}.

Selectivity and applicability for complex sample

To demonstrate the selectivity, amplified DNA of *Staphylococcus* (*S.aureus*) was selected as control DNA. As shown in Fig. 3, the selective binding to target DNA produced significantly increased fluorescence intensity, whereas the

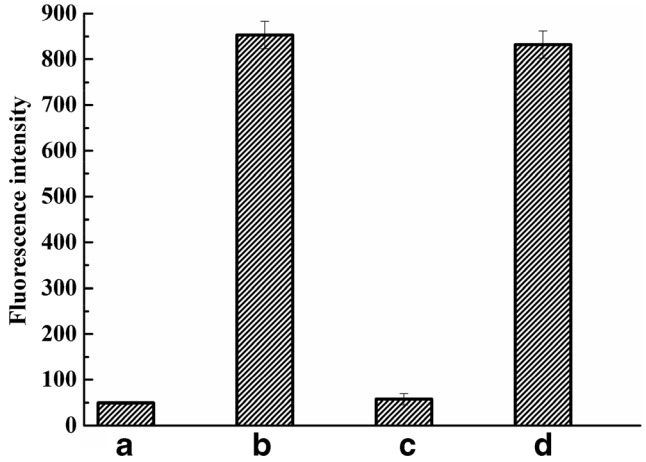


Fig. 3 Specificity analysis of detecting method based on Lysozyme reporter system with PCR products of (**a**) Blank E.coil sample, (**b**) 1×10^8 CFU·mL^{-1} E.coil, (**c**) 1×10^8 CFU·mL^{-1} S.aureus, (**d**) Mixed sample of 1×10^8 CFU·mL^{-1} E.coil and 1×10^8 CFU·mL^{-1} S.aureus

nonspecific binding to control DNA did not produce the conjunction between MBs and lysozyme, which led to a fluorescence intensity equivalent to the blank sample. At the same time, the results were also compared with those obtained by mixed samples, while the fluorescence intensity was slightly lower than that of the pure target sample. This indicates that the nonspecific binding plays no role in mixed sample application. In this way, the significant results demonstrated that the assay is highly selective for the target by discriminating against the control sample.

To further challenge the feasibility of this system in complex sample, we also attempted the target DNA detection in diluted human serum sample, using the same experimental procedures as those for target DNA detection in buffer solution. The spiked target DNA was detectable both in the diluted human serum and in buffer solution (Fig. 4), indicating very

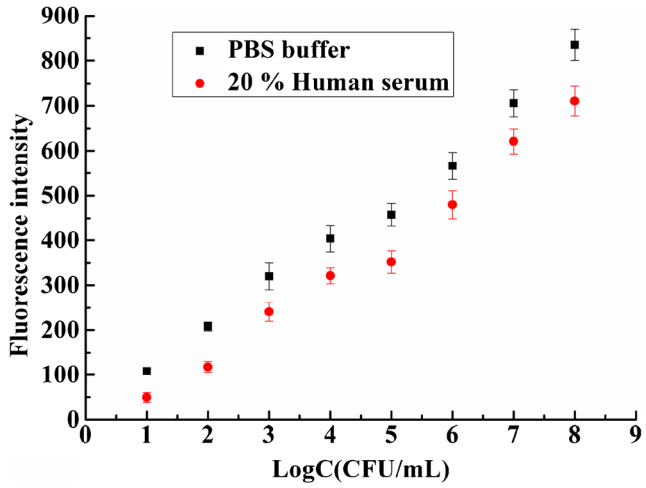


Fig. 4 Bacteria detection in 20 % human serum and PBS buffer based on Lysozyme reporter system

Table 1 Target bacteria DNA detected by the lysozyme reporter system in 20-fold-diluted serum samples

Sample	Added (CFU·mL^{-1})	Detected (CFU·mL^{-1})	Recovery (%)	RSD (%)[a]
1	1.0×10^3	0.902×10^3	90.02	5.73
2	1.0×10^4	1.110×10^4	111.0	4.81
3	1.0×10^5	0.987×10^5	98.70	5.02
4	1.0×10^6	1.075×10^6	107.5	6.10

[a] Each sample was repeated for three times and averaged to obtain the recovery and RSD values

little interference of complex buffer on this method. The recovery test was summarized in Table 1, with satisfied values between 90.02 % and 111.0 %, which indicated that the possible interference from the serum on target DNA detection was negligible.

Application in bacteria detection with colorimetry

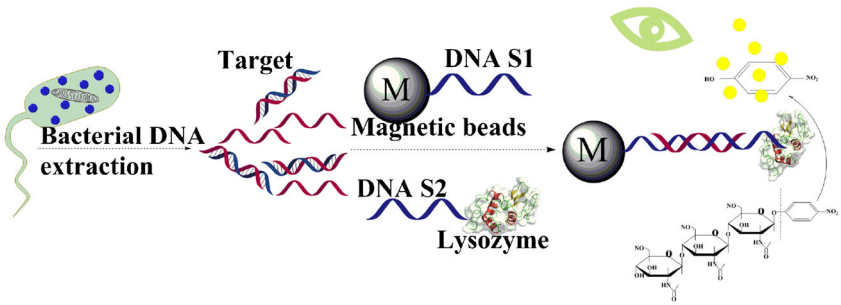
In order to further explore our methodology based on lysozyme-DNA conjugate, colorimetric detection without using fluorescence instrument is described. Colorimetric detection is convenient and effective in many labs with few resources, which can be monitored with bare eye [23]. Substrate 4-nitrophenyl-β-D-N, N′, N″-triacetylchitotriose (colorless) was catalyzed by lysozyme to generate Nitrophenol with yellow color (alkaline environment) in this study, which can be monitored by bare eye. Before bacteria detection with colorimetric system, experimental conditions for lysozyme colorimetric catalytic reaction was optimized, which was shown in Figure S3.

A schematic representation of the colorimetric system is presented in Scheme 2, and Fig. 5 shows the results for the detection of bacteria with this system. A blank test was carried out in the absence of target DNA. No color was formed because there was no lysozyme (that catalyze substrate) was available to bind to the MBs. However, after the introduction of target DNA extracted from 1×10^8 CFU·mL^{-1} bacteria, the resulted solution showed yellow color which can be easily differentiated from blank sample apparently. With decrease of bacteria concentrations, the yellow color gradually becomes lighter, resulting in final limited detecting concentration close to 1×10^3 CFU·mL^{-1} bacteria. The ability to detect with bare eye was further corroborated by measuring the absorbance with UV/Vis absorption. Increase of bacteria concentration also results in a clear increase in the absorbance at 395 nm, and the fluorescent intensity is linear to the logarithm of bacteria concentration (from 1×10^2 to 1×10^8 CFU·mL^{-1} bacteria), with a detected limit of 1×10^2 CFU·mL^{-1} *E. coli* (Fig. 5b, inset). The linear regression equation is Y = 0.0814 lgx-0.1081, with a correlation coefficient of 0.9966.

The above findings show that different concentrations of bacteria result in different colors. This implies that visual detection does not provide results as accurate as those of absorbance measurements, and therefore cannot be applied to precise quantification. However, compared with the conventional assays, it works in the most intuitive manner, which would be widely applicable in some cases need rapid positive detection or semi-quantitative detection.

Conclusion

Nowadays, more and more enzyme-labeled assay for bioanalysis appeared. Ordinarily enzyme-linked immunosorbent assays (ELISA) are based on horseradish peroxidase (HRP), alakine phosphatase (ALP). Other assays included portable operated, easy-observed assays, or some applications with enzyme rarely explored. For example, coupled with the presence of catalase, Stevens and his coworkers introduced a signal generation mechanism for biosensing with the bare eye [8]. Lu and his coworkers demonstrated a DNA assay linked to a commercially available personal glucose meter (PGM) by using the conversion of invertase [12]. To the best of our knowledge, Lysozyme as sensitive reporter for bacteria optical sensing described in this study has not yet been reported.



Scheme 2 Colorimetric sensing mechanism of bacteria detection with the bare eye using lysozyme as sensitive reporter

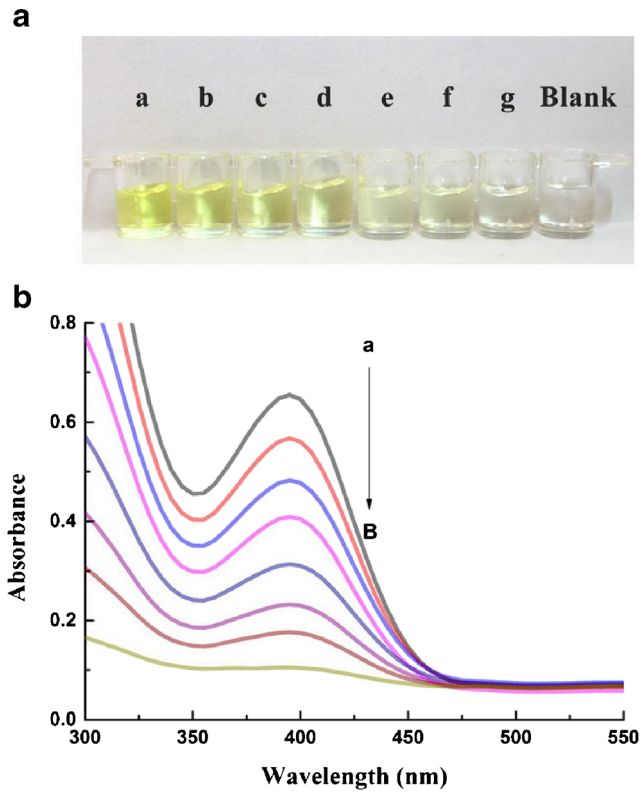


Fig. 5 **a** Bare detection of bacteria using lysozyme reporter system, **b** UV/Vis spectrum to PCR products obtained from different concentrations of E.coli with (a) 1×10^8, (b): 1×10^7, (c): 1×10^6, (d): 1×10^5, (e): 1×10^4, (f): 1×10^3, (g): 1×10^2 CFU·mL^{-1} E. coli and Blank

In summary, we described a simple and novel system for E.coli and S. aureus detection with lysozyme as a sensitive reporter and magnetic microbeads as carrier. This assay is based on the binding of target bacterial DNA to a capture probe immobilized on MBs. Lysozyme acts as a signal reporter that catalyzes the hydrolysis of a synthetic substrate to form a fluorescent product, resulting in the concentration of bacteria DNA tends to be proportional to fluorescence signal. This method is highly sensitive (down to 50 CFU·mL^{-1} bacteria) with multiple steps of signal amplification, including PCR amplification, Magnetic amplification and lysozyme catalytical amplification. In addition, the method exhibited excellent selectivity and applicability, being able to differentiate different bacteria target and used in complex buffer like human serum. Moreover, as lysozyme substrate is multiple, not only the fluorescence detection was evaluated. Colorimetric detection which can be detected with bare eye was also carried out, making it possible to apply this method to variable detection schemes. The use of lysozyme reporter provides new perspectives for lysozyme application and has a large potential in biosensing.

Acknowledgments This work was supported by the National Natural Science Foundation of China (41306072), National Key Basic Research Program of China (2014CB643304), Shandong Provincial Natural Science Foundation, China (BS2013HZ005 and ZR2014DQ009).

References

1. Chung HJ, Castro CM, Im H, Lee H, Weissleder R (2013) A magneto-DNA nanoparticle system for rapid detection and phenotyping of bacteria. Nat Nanotechnol 8(5):369–375
2. Yuan JL, Yu Y, Li C, Ma XY, Xia Y, Chen J, Wang ZP (2014) Visual detection and microplate assay for Staphylococcus aureus based on aptamer recognition coupled to tyramine signal amplification. Microchim Acta 181(3):321–327
3. Wan Y, Sun Y, Qi P, Wang P, Zhang D (2014) Quaternized magnetic nanoparticles-fluorescent polymer system for detection and identification of bacteria. Biosens Bioelectron 55:289–293
4. Ottesen EA, Hong JW, Quake SR, Leadbetter JR (2006) Microfluidic digital PCR enables multigene analysis of individual environmental bacteria. Science 314(5804):1464–1467
5. Wang YR, Stanzel M, Gumbrecht W, Humenik M, Sprinzl M (2007) Esterase 2-oligodeoxynucleotide conjugates as sensitive reporter for electrochemical detection of nucleic acid hybridization. Biosens Bioelectron 22(8):1798–1806
6. Song WL, Zhang Q, Xie XX, Zhang SS (2014) Fluorescence aptameric sensor for isothermal circular strand-displacement polymerization amplification detection of adenosine triphosphate. Biosens Bioelectron 61:51–56
7. Wang QB, Xu N, Lei JP, Ju HX (2014) Regulative peroxidase activity of DNA linked hemin by graphene oxide for fluorescence DNA sensing. Chem Commun 50(51):6714–6717
8. De la Rica R, Stevens MM (2012) Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat Nanotechnol 7(12):821–824
9. Xu ZL, Yin HS, Tian ZB, Zhou YL, Ai SY (2014) Electrochemical immunoassays for the detection the activity of DNA methyltransferase by using the rolling circle amplification technique. Microchim Acta 181:471–477
10. Li W, Ge JY, Zhao CZ (2015) Highly sensitive electrochemiluminescence determination of cholesterol utilizing a funcional electrode with a core-shell nanostructure. Luminescence 30(6):853–858
11. Khodakov D, Ellis A (2014) Recent developments in nucleic acid identification using solid-phase enzymatic assays. Microchim Acta 181:1633–1646
12. Xiang Y, Lu Y (2011) Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. Nat Chem 3(9):697–703
13. Fleming A (1922) One remarkable bacteriolytic element found in tissues and secretions. Proc R Soc B 93:306–317
14. Kao KC, Lin TS, Mou CY (2014) Enhanced activity and stability of lysozyme by immobilization in the matching nanochannels of mesoporous silica. J Phys Chem C 118(13):6734–6743
15. Yang Y, Hamaguchi KJ (1980) Hydrolysis of 4-methylumbelliferyl N-acetyl-chitotrioside catalyzed by hen and Turkey lysozymes pH dependence of the kinetics constants. Biochem 87(4):1003–1014
16. Jacks IJ, Kircher HW (1967) Fluorimetric assay for the hydrolytic activity of lipase using fatty acyl esters of 4-methylumbelliferone. Anal Biochem 21(2):279–285
17. Sang LC, Coppens MO (2011) Effects of surface curvature and surface chemistry on the structure and activity of proteins adsored in nanopores. Phys Chem Phys 13:6689–6698

18. Osawa T, Nakazawa Y (1966) Lysozyme substrate: chemical syntheis of p-nitrophenylO-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside and its reaction with lysozyme. Biochim Biophys Acta 130(1):56–63
19. Xiang Y, Lu Y (2012) Using commercially available personal glucose meters for portable quantificaiton of DNA. Anal Chem 84(4): 1975–1980
20. Lu J, Paulsen IT, Jin DY (2013) Application of exonuclease III-aided target recycling in flow cytometry: DNA detection sensitivity enhanced by orders of magnitude. Anal Chem 85(17):8240–8245
21. Zhou ZJ, Xiang Y, Tong AJ, Lu Y (2014) Simple and efficient method to purify DNA-protein conjugates and its sensing applicaitons. Anal Chem 86(8):3869–3875
22. Zeng Y, Wan Y, Zhang D, Qi P (2015) A novel magneto-DNA duplex probe for bacterial DNA detection based on exonuclease III-aided cycling amplification. Talanta 132:59–64
23. Kwon D, Joo J, Lee J, Park K-H, Jeon S (2013) Magnetophoretic chromatography for the detection of pathogenic bacteria with the naked eye. Anal Chem 85(15):7594–7598