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Antibiotic-affinity strategy for bioluminescent detection of viable Gram-positive bacteria using daptomycin as recognition agent

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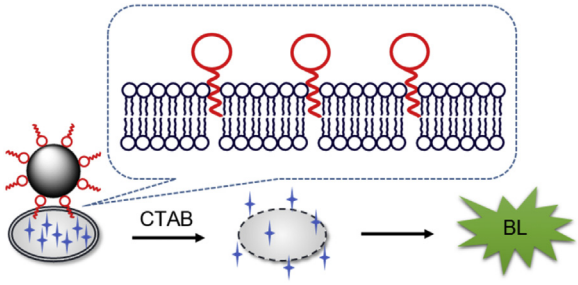
HIGHLIGHTS

- A bioluminescent protocol was developed for the detection of viable Gram-positive bacteria.
- Daptomycin was used to functionalize magnetic beads for Gram-positive bacteria capture.
- It could exclude the interference from both Gram-negative bacteria and dead Gram-positive bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

A bioluminescent method was proposed for rapid detection of viable Gram-positive bacteria based on a novel antibiotic-affinity strategy on a magnetic beads (MBs) platform. Daptomycin, a highly efficient lipopeptide antibiotic for Gram-positive bacteria, was used as a recognition agent to functionalize MBs. The daptomycin-functionalized MBs showed high capture and concentration efficiency for Gram-positive bacteria due to the strong binding between daptomycin and bacterial cell membrane in the presence of Ca^{2+} ion. The captured bacteria were lysed by hexadecyl trimethyl ammonium bromide solution, followed by a bioluminescent detection of the released intracellular adenosine triphosphate. Four Gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus epidermidis*, were detected as model bacteria by this method. Under the optimal conditions, the bacteria could be detected within a linear range of 1.0×10^2–3.0×10^6 CFU mL^{-1}, with a detection limit of 33 CFU mL^{-1}. The whole detection procedure could be completed within 20 min. Gram-negative bacteria and dead Gram-positive bacteria showed negligible interference to the detection of viable Gram-positive bacteria. The proposed method was successfully applied to quantify the amount of viable Gram-positive bacteria in cheese, milk, lake water, human urine and physiological saline injection with acceptable recovery values ranging from 75.0% to 120.0%. The strategy possessed some advantages such as high sensitivity, short assay time and simple operation, thus showed great promise for food hygiene, environment monitoring, clinical diagnosis and drug safety.

1. Introduction

As one of the leading causes of illness and death, infectious diseases impose great threat to public health and safety, particularly

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in most developing countries. Statistically, various bacterial infections are responsible for around one-third of global mortality [1]. Thus, it is of great importance to develop rapid, sensitive and facile methods for bacterial detection in such areas as food hygiene, environment monitoring, clinical diagnosis and drug safety.

The conventional detection based on bacterial culture and colony counting is still regarded as the gold standard for bacterial detection because of its ideal reliability [2]. Nevertheless, it requires tedious manipulation and time-consuming culture, making it unsuitable for rapid screening and point-of-care test. Some molecular biology techniques such as polymerase chain reaction (PCR) [3] and real-time PCR [4] have been developed as rapid detection methods for bacteria. These molecular biology based methods are extremely sensitive and shorten the whole procedure to several hours, but demand purified DNA samples and expertise in molecular biology [5]. Recently, a variety of molecular recognition based methods have been developed to solve the above mentioned problems. Various biological material, including antibody [6–8], bacteriophage [9,10], aptamer [11,12] and antimicrobial peptide [13,14], have been utilized in this mode to rapidly recognize bacterial cells. Nevertheless, most of these biological macromolecule based recognition agents suffer from deficiencies such as poor stability, high cost and varying performances among batches and sources. Therefore, additional efforts are still urgently needed to find cost-efficient and high stable micromolecular recognition agents suitable for resource-limited regions and harsh environment.

Daptomycin is a lipopeptide antibiotic produced as a fermentation end product of a soil bacterium *Steptomyces roseosporous* [15,16]. It is a cyclic molecule with 13 amino acids and a decanoyl fatty acyl side chain attached to the exocyclic N-terminal tryptophan residue [17]. Daptomycin shows rapid bactericidal activity against a variety of Gram-positive bacteria, notably against multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*) and vancomycin-resistant *Enterococcus* [18]. The action target of this antibiotic is the bacterial cell membrane. In the presence of Ca^{2+} ion, daptomycin inserts its hydrophobic tail into the cell membrane of Gram-positive bacteria, causing K^{+} ion leakage and subsequent membrane depolarization, thus leading to cell death without lysis [19–21]. Based on the unique mechanism, daptomycin has the potential to be used as a recognition agent for capturing Gram-positive bacteria.

Adenosine triphosphate (ATP) is a universal energy storage molecule existing in all viable bacteria, and its level in bacterial cells remains fairly constant [22]. Bioluminescence (BL) detection based on firefly luciferin-luciferase system has already been adopted for quantification of intracellular ATP level due to its extremely high sensitivity [23,24]. However, for real sample detection, this direct BL detection method trends to be interfered by complex sample matrixes.

Here, daptomycin-functionalized magnetic beads (dapt-MBs) were prepared to capture and concentrate Gram-positive bacteria from sample matrix utilizing the strong binding between daptomycin and bacterial cell membrane. After extraction of ATP in the cells of captured bacteria using hexadecyl trimethyl ammonium bromide (CTAB), the bacterial concentration was quantified by detecting ATP BL intensity. This method showed high sensitivity, facile manipulation, short detection time, and excluded the interference from Gram-negative bacteria, dead Gram-positive bacteria and sample matrix.

2. Experimental

2.1. Reagents and materials

Carboxy groups-coated magnetic beads (MBs) with a diameter of 3.0 μm were purchased from Biospes Co., Ltd. (China). Daptomycin, *N*-hydroxysuccinimide (NHS) and 2-(*N*-morpholino) ethanesulfonic acid (MES) were obtained from J&K Chemical Co., Ltd. (China). Tris (hydroxymethyl) aminomethane (Tris) and d-luciferin were supplied by Sigma-Aldrich Chemical Co., Ltd. (USA). Bovine serum albumin (BSA) was purchased from Gibco (USA). Fluorescein isothiocyanate (FITC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), CTAB and beta-cyclodextrin (β-CD) were all obtained from Aladdin Reagent Ltd. (China). Firefly luciferase was provided by Promega (USA). Luria-Bertani broth was purchased from Oxoid, Ltd. (USA). Dithiothreitol (DTT) and ethylenediaminetetraacetic acid (EDTA) were purchased from Chengdu Kelong Chemical Co., Ltd. (China). Strains of *S. aureus* (CCTCC AB 91093), *Streptococcus mutans* (*S. mutans*) (CCTCC AB 99010) and *Bacillus subtilis* (*B. subtilis*) (CCTCC AB 90008) were all obtained from China Center for Type Culture Collection (China). Strains of *Staphylococcus epidermidis* (*S. epidermidis*) (GIM 1.444), *Escherichia coli* (*E. coli*) (GIM 1.223), *Pseudomonas aeruginosa* (*P. aeruginosa*) (GIM 1.220), *Salmonella typhimurium* (*S. typhimurium*) (GIM 1.237) and *Shigella dysenteriae* (*S. dysenteriae*) (GIM 1.236) were provided by Guangdong Microbiology Culture Centers (China). Milk was purchased from the local supermarket. Lake water was collected from Chongde Lake in Southwest University campus. Human urine was provided by a healthy adult volunteer. Physiological saline injection was obtained from the local pharmacy. Polystyrene 96-well microplates were provided by Corning Inc. (USA).

Dilution buffer for bacteria was 25 mM Tris-HCl buffer (pH 7.4) containing 5.0 mM $CaCl_2$. Coupling buffer for preparation of dapt-MBs was 50 mM MES buffer at pH 5.2. Washing buffer was 25 mM Tris-HCl buffer at pH 7.4. ATP extraction buffer was 25 mM Tris-HCl buffer (pH 7.4) containing 5.0 mM CTAB and 2.5 mM EDTA. BL reagent solution was 25 mM Tris-HCl buffer (pH 7.4) containing 0.075 mg mL^{-1} luciferase, 0.25 mg mL^{-1} d-luciferin, 25 mM Mg^{2+}, 2.5 mM EDTA, 2.5 mM DTT and 2.5 mg mL^{-1} BSA.

2.2. Instrumentations

BL signals were collected using a MPI-A CL analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd, China) equipped with a photomultiplier operated at −800 V. Ultrapure water was prepared by an ELGA PURELAB classic system (France). Scanning electron micrographs were obtained with an S-3000N scanning electron microscope (SEM) (Hitachi Instrument Co., Ltd., Japan). Fluorescent micrographs of *S. mutans* were obtained using an A1+ confocal laser microscope (Nikon Instruments Co., Ltd., Japan). Treatment of bacteria was conducted in a biosafety cabinet (biosafety level II) (Suzhou Antai Airtech Co., Ltd., China).

2.3. Bacterial culture and counting

All bacteria strains were cultivated overnight in Luria-Bertani broth at 37 °C with constant shaking till the optical density value at 600 nm (OD600) reached 1.0. The bacterial cultures were centrifuged at 5000 rpm for 5 min to remove the culture media. The collected pellets were resuspended and diluted to the desired concentration in dilution buffer. Bacterial cell number was quantified using a conventional agar plate counting protocol, and the results were converted to OD600 values.

2.4. Preparation of dapt-MBs

Daptomycin was covalently bound to the surface of carboxy groups-coated MBs by an EDC/NHS amidization reaction. Briefly, 1.0 mL of carboxy groups-coated MBs suspension was washed