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Applications of copolymer for rapid identification of bacteria in blood culture broths using matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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ABSTRACT

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used to identify pathogens in blood culture samples. However, sample pretreatment is needed for direct identification of microbes in blood culture bottles. Conventional protocols are complex and time-consuming. Therefore, in this study, we developed a method for collecting bacteria using polyallylamine–polystyrene copolymer for application in wastewater treatment technology. Using representative bacterial species *Escherichia coli* and *Staphylococcus capitis*, we found that polyallylamine–polystyrene can form visible aggregates with bacteria, which can be identified using MALDI-TOF MS. The processing time of our protocol was as short as 15 min. Hemoglobin interference in MALDI spectra analysis was significantly decreased in our method compared with the conventional method. In a preliminary experiment, we evaluated the use of our protocol to identify clinical isolates from blood culture bottles. MALDI-TOF MS-based identification of 17 strains from five bacterial species (*E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *S. aureus*, and *S. capitis*) collected by our protocol was satisfactory. Prospective large-scale studies are needed to further evaluate the clinical application of this novel and simple method of collecting bacteria in blood culture bottles.

1. Introduction

In 1975, John and Fenselau (1975) reported the first use of mass spectrometry (MS) to identify bacteria. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS has increasingly been used to identify microorganisms including Gram-negative and -positive rods (Camara and Hays, 2007; Ryzhov et al., 2000), Gram-positive cocci (Edwards-Jones et al., 2000), and yeast (Spanu et al., 2012). MALDI-TOF MS can be used to generate protein fingerprint signatures from whole bacterial cells (Biswas and Rolain, 2013; Patel, 2015). Using various algorithms, bacteria can be rapidly identified by comparing these fingerprints with those of reference spectra (Del Chierico et al., 2014; Schulthess et al., 2013; Sogawa et al., 2012).

Blood culture is the most important method for diagnosing bloodstream bacterial infections. Pretreatment is needed for the direct identification of microbes from blood cultures, and the performance of several in-house methods and commercial kits have been reported (Fothergill et al., 2013; Meex et al., 2012). Conventional protocols, however, are complex and involve extensive hands-on time (Farina et al., 2015). Such techniques are also costly (Chen et al., 2013) and subject to interference from residual blood debris including hemoglobin-related proteins (Buchan et al., 2012; Martiny et al., 2012).

In the field of wastewater management, studies have investigated the use of electrical coupling between positively charged particle surfaces and negatively charged bacterial surfaces (Terada et al., 2006). Accordingly, polyallylamine, which has a hydrophobic group attached to the metal, can form a bond between its hydrophobic group and low molecular weight particles as a method of precipitation (Ueda

Abbreviations: CHCA, α-cyano-4-hydroxycinnamic acid; GFP, green fluorescent protein; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MS, mass spectrometry

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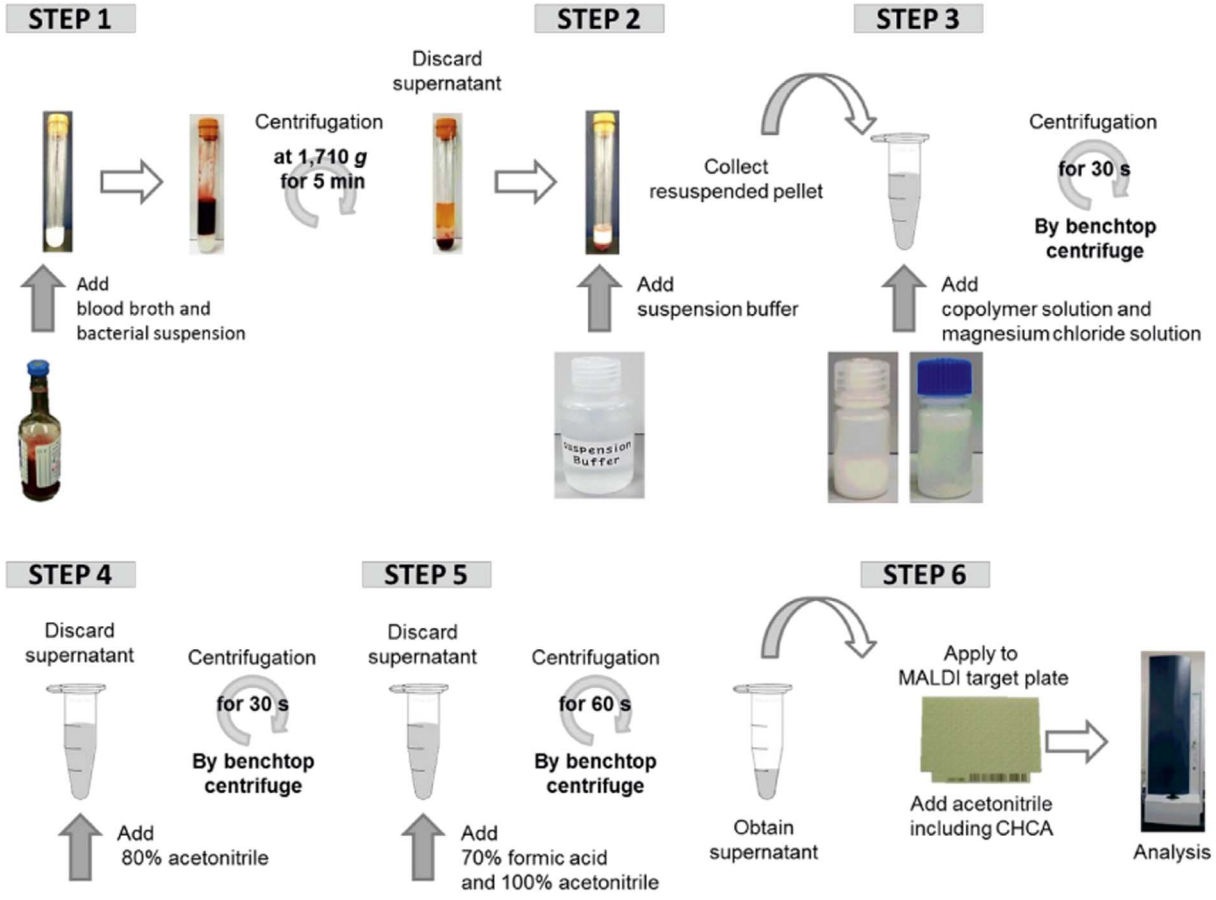

Fig. 1. Workflow of the protocol using copolymer.

and Harada, 1968).

The present study aimed to apply polyallylamines used in wastewater management to effectively collect bacteria in blood culture broths, a process that may, in turn, simplify pretreatment processes and decrease the processing time compared with conventional methods. We herein report on a prototype polyallylamine copolymer to collect bacteria in blood culture broths for MALDI-TOF MS-based identification.

2. Materials and methods

2.1. Preparation of model samples

Escherichia coli (pGLO™ Bacterial Transformation Kit; Bio-Rad Laboratories, Hercules, CA, USA) and *Staphylococcus capitis* (ATCC 35661; Kanto Chemical, Tokyo, Japan) were used as representative bacteria. These organisms were cultured overnight in liquid culture medium (BBL™ Brain Heart Infusion (BHI); Becton, Dickinson, Franklin Lakes, NJ, USA) at 37 °C. The bacterial suspension was adjusted to a final concentration of 1×10^9 colony forming units (CFUs)/mL in saline based on the absorbance at a wavelength of 550 nm, according to the McFarland standard. Blood broth was prepared by adding 10 mL of blood to BACTEC Plus Aerobic bottles (Becton Dickinson). The blood (whole blood with 3% citric acid as the anticoagulant) used in this experiment was obtained from the Japanese Red Cross Society with approval by the public offering based on the "Guidelines on the use of donated blood in R & D" of the Japanese Ministry of Health, Labour and Welfare.

2.2. Selection of appropriate polyallylamine and reaction conditions

The bacterial suspension was diluted with saline to a concentration of 1×10^8 CFU/mL. Polyallylamine was dissolved in 1 M Tris-glycine buffer. Then, 200 µL of 5 wt% polyallylamine, 200 µL of crosslinking agent buffer, and 1 mL of the sample were mixed using a vortex mixer at high speed and centrifuged at 2000 × *g* for 30 s using a Chibitan R benchtop centrifuge (Merck Millipore, HE, Darmstadt, Germany). The presence or absence of aggregates was confirmed by macroscopic inspection. We then examined whether various polyallylamines including primary, secondary, and tertiary polymers, as well as a copolymer of polyallylamine and polystyrene (120–140 nm), formed visible aggregates with bacteria. Additionally, we screened for the most suitable metal compounds for crosslinking and the optimal pH for the reaction, as described in Supplementary Fig. 1. To select metal ions for crosslinking, the bacterial suspension and saline solution were used as samples. The bacterial suspension was used to confirm whether the copolymer could capture and aggregate bacteria, and the saline solution was used to confirm self-aggregation. The supernatant generated after mixing was diluted 1:10 with purified water and the absorbance at 600 nm was measured. The difference in absorbance between the mixture incorporating saline and that incorporating the bacterial suspension was considered to indicate the amount of polymer that captured bacteria. Larger differences in absorbance were interpreted to indicate decreased self-aggregation and increased bacterial capture capacity.

2.3. Interaction between bacteria and the polyallylamine–polystyrene copolymer

E. coli cells were transfected with green fluorescent protein (GFP)-