Available online at www.sciencedirect.com
ScienceDirect
Sensors and Actuators B 127 (2007) 335–340
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Gold nanoparticle-based immunochromatographic assay for the detection of *Staphylococcus aureus*

Su-Hua Huang *

Department of Biotechnology and Bioinformatics, College of Arts and Sciences, Asia University, No. 500, Lioufeng Road, Wufeng, Taichung 41354, Taiwan, ROC

Received 28 January 2007; accepted 17 April 2007
Available online 24 April 2007

Abstract

An analytical system of immunochromatographic assay based on gold nanoparticles was developed for the detection of *Staphylococcus aureus*. Staphylococcal protein A, a cell wall protein of *S. aureus*, has the ability to interact with several host components, possibly indicating a role as a virulence factor in *S. aureus* infections. The assay was constructed in the form of sandwich by using anti-protein A IgG with two distinct specificities. One anti-protein A IgG was immobilized in a defined detection zone on a porous nitrocellulose membrane, while the other anti-protein A IgG was conjugated with gold nanoparticles. The sample flows along the porous membrane by capillary action past the anti-protein A IgG in the detection zone, binding the particles that to which surface protein A was already bound to their surface, yielding a red color. The rapid observation of results directly by the naked eye ensures the convenience of performing bioassays on field. As the results, the sensitivity in the immunochromatographic test of 306 *S. aureus* strains were 100% and the specificity of 44 non-*S. aureus* strains were 96.0 to 100%. Twelve processed food samples inoculated artificially with 0.9, 1.2, 2.4, and 6 CFU/g of *S. aureus* and all yielded positive results in the immunochromatographic test. As compared to the conventional culture methods which take 5 to 6 days to complete, the immunochromatographic test can detect low numbers of *S. aureus* in processed foods with a total analytical time of only 25 h.

Keywords: Immunochromatographic assay; *Staphylococcus aureus*; Anti-protein A IgG; Gold nanoparticles; Nitrocellulose membrane

1. Introduction

Staphylococcus aureus food poisoning is a disease characterized by sudden onset of symptoms including nausea, vomiting, abdominal cramps, and diarrhea within 2–6 h after ingestion of toxin-contaminated foods [1]. *S. aureus* can produce several types of enterotoxins that cause gastroenteritis, which is a major food-borne disease in most countries [2]. Hence, foods contaminated with enterotoxigenic *S. aureus* are potential sources of enterotoxins if stored at an unsuitable temperature, at which the microorganism can grow and synthesizes enterotoxins. Thus, a rapid screening method for *S. aureus* is important.

Foods contain relatively few *S. aureus* bacteria, so an enrichment procedure to isolate of *S. aureus* is normally performed [3,4]. Following enrichment, selective Baird–Parker agar is often employed to isolate suspect colonies, and a coagulase test is performed to identify *S. aureus*. Moreover, if a definite coagulase test (4+) is not obtained, then several ancillary tests be performed [3,4]. The complete protocol may tests take up to 5 or 6 days.

Several methods have been reported for rapid method of identification and discrimination of *S. aureus* in foods, such as, flow cytometry [5], latex agglutination test [6], enzyme-linked immunosorbent assay [7], Fourier transform infrared (FTIR) spectroscopy [8], surface plasmon resonance biosensors [9] and Electrochemical sensor [10]. However, the latex agglutination test and FTIR spectroscopy can only be performed on pure cultures, so 2 to 3 days are required before this test can be performed.

If a specific metabolite of *S. aureus* can be used to indicate the presence of *S. aureus* in an enrichment broth, the time required to detect the bacterium can be greatly reduced. Protein A seems to be a good candidate for this purpose. Protein A, a 42 kDa protein that is covalently anchored to the cell wall of most *S. aureus* strains, was first characterized as a protein with the abil-

* Tel.: +886 4 23323456x1865; fax: +886 4 22215185.
E-mail address: shhuang@asia.edu.tw.

0925-4005/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2007.04.027

ity to interact with human IgG in a non-antigen specific manner [11]. Protein A contains five repeated units, each with the ability to mediate binding to the Fc portion of several mammalian IgG subclasses. Also, each of the repeated units has affinity for Fab domains encoded by some specific immunoglobulin heavy chain gene families [12]. Protein A is a specific product of *S. aureus* and about 99% of the bacterial strains contain this protein [13,14]. Therefore, this work was conducted to determine the feasibility of the use of anti-protein A IgG to detect rapidly *S. aureus*. Moreover, a specific enrichment broth was formulated for this purpose.

This report describes the preparation of anti-protein A IgG antibodies using labels of gold nanoparticles and the development of immunochromatographic assay based on gold nanoparticles to detect *S. aureus*.

2. Experimental

2.1. Microorganisms and culturing conditions

The 350 strains used in this study are listed in Table 1. Among the tested 306 strains of *S. aureus*, 66 were obtained from the BCRC (Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan), and the other 240 were isolated from various foods by the Lab of Food Microbiology (Food Industry Research and Development Institute, Hsinchu, Taiwan). Other 44 strains representing 15 species bacteria were also from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). All strains were grown and maintained on tryptic soy agar (TSA) slants, except for that of *Vibrio parahaemolyticus* which was maintained on TSA with 3% sodium chloride (NaCl). In performing immunochromatographic assay of protein A from various strains, fresh cultures grown overnight on TSA were inoculated into staphylococcal selective broth (SSB) [5] and incubated at 37 °C for 24 ± 2 h.

Table 1
Secretion of protein A by different bacterial strains grown in SSB at 37 °C for 24 h

Microorganism (staphylococcal selective broth)	No. of strains tested	No. of positive tests	No. of negative tests
Staphylococcus aureus	306	306	0
S. cohnii subsp. cohnii	3	0	3
S. intermedius	3	0	3
S. warner	3	0	3
S. hominis	3	0	3
S. haemolyticus	2	0	2
S. epidermidis	3	0	3
S. xylosus	2	1	1
S. lentus	3	0	3
S. saprophyticus	3	0	3
Vibrio parahaemolyticus	2	0	2
Micrococcus varians	5	0	5
Micrococcus luteus	3	0	3
E. coli	3	0	3
Corynebacterium glutamicus	1	0	1
Bacillus subtilis	5	0	5

2.2. Preparation and purification of anti-protein A IgG

Detailed procedures were described in a previous report [7]. Briefly, commercial protein A (Pharmacia, Uppsala, Sweden) was further purified by electrophoresis using sodium dodecyl sulfate polyacrylamide gel (3 mm thick). The band that corresponds to protein A (molecular weight 42 kDa) was excised and crushed into fine particles with a hand homogenizer. The crushed gel which contained approximately 1 mg protein A was emulsified with 4 ml of Freund's incomplete adjuvant. Two of the emulsified antigen was injected subcutaneously at many sites on the back of each New Zealand white rabbit. The rabbits were boosted twice at 3-week intervals in the same way. Seven days after the final injection blood was collected from the ear vein. The IgG fraction of the antisera was purified by diethylaminoethyl (DEAE) ion exchange chromatography [15].

2.3. Preparation of gold nanoparticles

The 20 nm gold nanoparticles were prepared by a modified citrate reduction method [16]. Briefly, 200 ml of 0.01% $HAuCl_4$ solution was brought to a rolling boil with vigorous stirring. Rapidly adding 2.6 ml of 1% sodium citrate solution to the vortex of the solution changed its color from pale yellow to burgundy. Boiling was continued for 10 min, the heating mantle was then removed, and stirring was continued for a further 15 min. After the solution reached room temperature, it was filtered through a 0.8 μm Gelman membrane filter. The approximate concentration of the gold nanoparticles was determined by measuring the particle size by Hitachi S-700 transmission electron microscopy (TEM) at 75 kV.

2.4. Conjugation of antibody and gold nanoparticles

The gold nanoparticles were coated with anti-protein A IgG according to the procedure given in a previous report [17]. Briefly, 10 ml of gold nanoparticles was quickly added to 1 ml of anti-protein A IgG solution with vigorous stirring and the mixture was incubated at room temperature for 5 min. The concentration of protein in the anti-protein A IgG used was increased from 15 to 125 mg/ml. Optimal condition of pH and antibody concentration for the coating was determined by measuring the absorbance at wavelength of 580 nm, i.e., A_{580}. For antibody was obtained in the pH range 7–8. In contrast, there is a minimal antibody concentration approximately 25 mg/ml. After incubation, the coated gold nanoparticles were stabilized by adding 113 μl of bovine serum albumin (10%) to a concentration of 0.1% (w/v). The labeled conjugate was centrifuged at 8000 × *g* for 20 min and washed five times in 1 ml of buffer solution which contained 0.1% bovine serum albumin. The final volume of the labeled conjugate was 1 ml. The labeled conjugate was stored at 4 °C.

2.5. Preparation of membrane with immobilized antibody

The labeled antibody–analyte complex for signal generation was captured after the antibody had been immobilized in a particular area of the nitrocellulose membrane. The surface of the