A Research Note

Modification of the Conventional Procedure for the Test of Staphylococcal Coagulase

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ABSTRACT

The coagulase test is routinely used for the confirmation of suspect Staphylococcus aureus on Baird-Parker agar (BPA). Overnight (18 to 24 h) preincubation of suspect colonies in brain heart infusion (BHI) broth is generally practiced prior to the coagulase test. In order to shorten the test protocol, different preincubation times of S. aureus in BHI were evaluated. Stock cultures (257 strains) of S. aureus were subcultured on BPA, and single colonies of each strain on BPA were analyzed for coagulase activities by the conventional procedure except that the preincubation times in BHI were varied (0, 4, and 24 h). The formation of a clot was examined at 2-h intervals over a 6-h period and at 24 h, and any degree of clot formation (1+ to 4+) was considered a positive reaction. Low sensitivities were found for tests without preincubation in BHI prior to the enzyme test. However, for 4- and 24-h preincubation, there was no difference in the degree of clot formation and in the sensitivities of the coagulase tests with incubation periods from 6 (98.1%) to 24 h (99.6%). Compared with the conventional procedures which may need two days, the modified protocol (4-h preincubation in BHI) can identify a large majority (97.7–98.1%) of suspect S. aureus on BPA within one working day.

Key words: Staphylococcus aureus, coagulase test

Staphylococcus aureus can produce several types of enterotoxins causing gastroenteritis, which is a major food-borne disease in most countries of the world (7). Although protein A is a better marker than coagulase and thermostable nuclease for the identification of S. aureus (6), it has no enzyme activity and can only be analyzed by immunological methods. Therefore, the Association of Official Analytical Chemists International (AOAC International), the Bacteriological Analytical Manual (BAM) and the American Public Health Association (APHA) all recommend the coagulase test for identification of S. aureus from foods (1, 2, 8).

To perform the test, suspect colonies on Baird-Parker agar (BPA) are transferred to a small volume of brain heart infusion (BHI) broth, incubated overnight (18 to 24 h), and then tested for coagulase activity over an incubation period of 6 h. Although the coagulase test is simple and reliable, overnight procedures are required to identify suspect S. aureus on BPA.

Several immunological methods have been developed for the rapid detection or identification of S. aureus from foods, including flow cytometry (3), a latex agglutination test (4) and an enzyme-linked immunosorbent assay (5). In addition to the above methods, optimization (or modification) of the conventional procedures also may have potential to shorten the identification time for S. aureus.

The purpose of this study was to evaluate the feasibility of shortening the preincubation time in BHI prior to the coagulase test for the identification of S. aureus. It was hoped that the identification of suspect S. aureus on BPA could be completed within one working day.

MATERIALS AND METHODS

Microorganisms

Among the 257 strains of S. aureus tested, 137 were obtained from the Culture Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan), and the other 120 were isolated from various foods (meat, vegetables, frozen meat, and fish products) by the Food Microbiology Section, Food Industry Research and Development Institute. All strains were maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) slants before analysis.

Coagulase test

Overnight fresh cultures on TSA were streaked on BPA (Difco) and incubated 44 to 48 h at 35°C. Isolated colonies of each strain on BPA were used for the coagulase test following the conventional protocol (1, 2) except that the preincubation times in BHI prior to the enzyme assay were varied (0, 4, or 24 h). Following the addition of EDTA-plasma (Difco) (0.5 ml) to the BHI culture broth (0.2 ml), the tubes were incubated at 37°C and the formation of a clot in each tube was examined at 2-h intervals over a 6-h period and at 24 h. Four types (1+ to 4+) of coagulase reactions were recognized (8), and any degree of clot formation (1+ or above) was considered a positive reaction as recommended.

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by AOAC International (1). Test sensitivity was defined as the numbers of coagulase-positive strains divided by total strains of *S. aureus* tested (9).

**RESULTS AND DISCUSSION**

Table 1 shows the results of coagulase tests of 257 strains of *S. aureus*. Low sensitivities were found for tests without preincubation in BHI prior to the enzyme test. Without preincubation, the test sensitivities were only 39.2 (96/257), 65.8 (169/257), 76.3 (196/257) and 92.2% (237/257), respectively, after 2, 4, 6, and 24-h incubations following the addition of rabbit plasma to the BHI broth. It was evident that preincubation in BHI was necessary to avoid false negatives. In general, two types of coagulase (cell bound and cell free) are recognized (10). Free coagulase is generally demonstrated by plasma clotting via the tube test whereas bound coagulase is demonstrated by bacterial cell clumping via the slide test. It seemed that a preincubation period was necessary for sufficient bacterial growth and secretion of the free enzyme for the coagulase test.

AOAC International (1), BAM (2), and APHA (8) all recommend an 18 to 24 h preincubation in BHI before performing the coagulase test; however, as shown in Table 1, the test sensitivity was almost the same when the preincubation time was reduced to 4 h. For 4 h of preincubation, the sensitivities of coagulase tests were 96.9 (249/257), 97.7 (251/257), 98.1 (252/257), and 99.6% (256/257), respectively, after 2, 4, 6, and 24 h of incubation following the addition of plasma. Compared with the short preincubation protocol (4 h), the long protocol (24 h) only had a very small increase (0.4%) in test sensitivity under the same incubation times (2 and 4 h) of the coagulase test (Table 1). However, the test sensitivities were the same if the incubation time of the coagulase test was extended to 6 h (98.1%) and 24 h (99.6%). In addition, the 4-h preincubation protocol did not affect the “firmness” of the plasma clot during the coagulase test. For the 4-h preincubation in BHI, 7 and 2 strains, respectively, demonstrated weak reactions (<3+) after 4- and 24-h incubations in the presence of plasma. However, for the 24-h preincubation in BHI, 13 and 4 strains, respectively, displayed weak reactions after 4 and 24 h of incubation. Therefore, compared to the short protocol (4 h of preincubation in BHI), the long protocol (18 to 24 h in BHI) did not increase the percentage of strains showing 3+ or 4+ coagulase activity. Following the 4-h preincubation protocol, the positive rates (97.7 to 98.1%) of coagulase tests with an incubation period from 4 to 6 h were very close to those of the previous report (6), in which a sensitivity of 97.7% was found after testing 213 strains of *S. aureus*.

In view of the above results and from a viewpoint of practicability, it seems that 4 h appears to be sufficient for both preincubation in BHI and for the coagulase test. A large majority (97.7%) of *S. aureus* could be identified by following the short protocol. Therefore, in contrast to the conventional procedures which may take as long as 2 days to identify suspect *S. aureus* on BPA, the modified protocol can be completed within one working day (8 h) without the sacrifice of test sensitivities to a large degree. However, a small percentage (1.5%) of *S. aureus* strains displayed positive coagulase reactions only after 24 h of incubation in the presence of plasma. Therefore, instead of an incubation period of 6 h, as recommended by AOAC International, BAM, and APHA, a reading of coagulase activity at 24 h is recommended for coagulase-negative strains. After testing 49 strains of *Staphylococcus* spp. other than *S. aureus*, an increase in false-positives was not found for prolonged incubation in the plasma (data not shown).

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**REFERENCES**