Polymyxin-Coagulase-Deoxyribonuclease-Agar: a Selective Isolation Medium for Staphylococcus aureus

D. S. ORTH and A. W. ANDERSON
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

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Fibrin halos developed around coagulase-positive Staphylococcus aureus colonies, and deoxyribonuclease production was detected by using a developing solution containing 1 mg of methyl green per ml.

The production of staphylococcal deoxyribonuclease is believed to be highly correlated with that of coagulase (1, 3, 4, 6, 11). The occurrence of potentially pathogenic, coagulase-negative staphylococci was reported by Grossgebauer et al. (5). Since not all pathogenic staphylococci produce coagulase, a selective medium which is capable of detecting coagulase and deoxyribonuclease may provide more certain identification of pathogenic staphylococci than the use of either test alone. This report compares the efficacy of Polymyxin-Coagulase-Deoxyribonuclease-Agar (PCDA), Polymyxin-Coagulase-Mannitol-Agar (PCMA), and Vogel-Johnson Agar (VJ) in recovering Staphylococcus aureus seeded into frozen food samples.

PCDA was prepared by adding the PCMA coagulase test system and polymyxin (9) to DNase Test Agar (BBL). After growth, the PCDA plates were flooded with 5 ml of 1 mg of methyl green (MG; Allied Chemical Corp., New York, N.Y.) per ml in 0.1 M sodium citrate buffer at pH 7.0. The MG developing solution was decanted from the surface of the plates after 1 min, and the plates were allowed to stand for 1 hr at room temperature for the color reaction to develop. Deoxyribonuclease activity was indicated by a zone of fading of the green staining around S. aureus colonies, whereas the background remained green. The deoxyribonuclease reaction was best seen with a white background.

Kurnick (7) demonstrated that MG preferentially stains polymerized deoxyribonucleic acid. The detection of deoxyribonuclease on media containing MG has been reported (8, 10). Adding as little as 0.002 μg/MG, along with 75 μg of polymyxin B, per ml proved to be too inhibitory for quantitative recovery of S. aureus from seeded food samples. This problem was overcome by elimination of MG from the medium during growth and flooding the plates with an MG solution after development of the colonies. The MG developing reagent utilized a citrate buffer to avoid the possibility of interfering with the coagulase reaction. Care must be exercised in decanting the MG solution so as to avoid contamination. Suspect colonies may be picked before flooding the plates if characterization of the isolates is desired. Although zones of deoxyribonuclease activity were evident within 1 hr after adding the dye, fading of the green around the deoxyribonuclease-positive colonies continued so that optimal color development took several hours.

The Staphylococcus cultures were grown in 5 ml of Brain Heart Infusion Broth (Difco) for 48 hr at 37°C, and 0.1 ml of each culture was inoculated into the frozen food products indicated in Table 1. The seeded samples were frozen and stored at −15°C for 48 hr. After thawing, serial 10-fold dilutions in Butterfield’s phosphate buffer (2) were spread-plated onto PCMA, PCDA, and VJ. The plates were incubated at 37°C.

The selectivity of PCDA was similar to that of PCMA since both media employed the same concentration of polymyxin B. Both PCDA and PCMA allowed sufficient growth for coagulase to form around the colonies of several of the test strains by the 15-hr reading. No colonies were visible on the VJ plates at this time. Most strains of S. aureus gave slightly larger colonies on PCMA than on PCDA by the 48-hr reading. Based on the samples examined, the recovery of S. aureus by PCMA and PCDA was greater...
than that of VJ at the 95% significance level (Table 1).

The usefulness of the dual-test media was shown in the recovery of the coagulase-negative *S. aureus*. Test procedures designed to detect only coagulase-positive staphylococci would fail to indicate the presence of this organism. Although recovered at a lower dilution than the coagulase-positive staphylococci, the coagulase-negative *Staphylococcus* was detected in the assay media on the basis of mannitol-fermentation on PCMA, deoxyribonuclease activity on PCDA, and growth and mannitol-fermentation on VJ.

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**TABLE 1. Comparison of the recovery of *Staphylococcus aureus*, seeded into frozen food samples, by using Polymyxin-Coagulase-Mannitol Agar (PCMA), Polymyxin-Coagulase-Deoxyribonuclease-Agar (PCDA), and Vogel-Johnson Agar (VJ)**

| Food sample               | ATCC culture(s) used to seed food sample | Recovery of *S. aureus* at 48 hr |
|---------------------------|-----------------------------------------|----------------------------------|
|                           | PCMA                                    | PCDA                             | VJ                                |
| Beef pie                  | 19095 and 19139                         | $80 \times 10^6$                 | $120 \times 10^6$                 | $30 \times 10^6$ |
|                           | 19139 and 23235                         | $160 \times 10^6$                 | $150 \times 10^6$                 | $145 \times 10^6$ |
| Chicken à la king         | 12600 and 13565                         | $200 \times 10^6$                 | $210 \times 10^6$                 | $200 \times 10^6$ |
|                           | 12600                                   | $76 \times 10^6$                  | $80 \times 10^6$                  | $52 \times 10^6$  |
| Chicken pie               | 19193 and 23235                         | $280 \times 10^6$                 | $220 \times 10^6$                 | $90 \times 10^6$  |
|                           | 8094 and 14458                          | $160 \times 10^6$                 | $130 \times 10^6$                 | $103 \times 10^6$ |
| French fried potatoes     | 13565                                   | $56 \times 10^6$                  | $71 \times 10^6$                  | $58 \times 10^6$  |
|                           | 13565 and 13566                         | $170 \times 10^6$                 | $150 \times 10^6$                 | $113 \times 10^6$ |
| Shredded hash brown potatoes | 14458                               | $50 \times 10^6$                  | $90 \times 10^6$                  | $56 \times 10^6$  |
|                           | 13566                                   | $180 \times 10^6$                 | $240 \times 10^6$                 | $200 \times 10^6$ |
| French onion rings        | 13566 and 14458                         | $220 \times 10^6$                 | $180 \times 10^6$                 | $290 \times 10^6$ |
|                           | 14458 and 19095                         | $140 \times 10^6$                 | $90 \times 10^6$                  | $71 \times 10^6$  |
| Shredded potato patties   | 8094 and 12600                          | $120 \times 10^6$                 | $120 \times 10^6$                 | $104 \times 10^6$ |
|                           | 8094                                    | $100 \times 10^6$                 | $220 \times 10^6$                 | $97 \times 10^6$  |
| Sliced turkey with gravy  | 19139                                   | $100 \times 10^6$                 | $70 \times 10^6$                  | $50 \times 10^6$  |
|                           | Coagulate-negative S. aureus*           | $0.77 \times 10^{4b}$             | $0.12 \times 10^{4b}$             | $0.47 \times 10^{4b}$ |
| Turkey pie                | 19095 and 19139                         | $140 \times 10^6$                 | $90 \times 10^6$                  | $71 \times 10^6$  |
|                           | 19095                                   | $75 \times 10^6$                  | $85 \times 10^6$                  | $54 \times 10^6$  |

* Coagulase-negative and deoxyribonuclease-positive *S. aureus* was obtained from the Oregon State University culture collection. This strain was recovered from a food incriminated in a food-poisoning outbreak and produced food-poisoning symptoms in 8-week-old kittens.

* Enumeration of the numbers of coagulase-negative *S. aureus* was based on mannitol-fermentation on PCMA, on deoxyribonuclease activity on PCDA, and on growth on VJ.