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NOTES

New Modification of Willis and Hobbs' Method for Identification of *Clostridium perfringens*

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A modification of the medium of Willis and Hobbs is described. All strains of *Clostridium perfringens* grown on it gave a positive lecithinase reaction. Some gave a negative reaction in the original medium.

The medium of Willis and Hobbs (WH) (3) can be used for the identification of *Clostridium perfringens*. Both the lecithinase reaction in egg yolk and lactose fermentation can be observed, although some atypical strains are lactose positive and lecithinase negative (4). We have made similar observations with some of our strains, which we have overcome by adding yeast extract (0.45%), $Na_2HPO_4 \cdot 7H_2O$ (0.5%), sodium thioglycolate (0.1%), and proteose-peptone (1.5%) to WH medium (3).

Four strains of *C. perfringens* were used: strains C and III from food-poisoning outbreaks and strains 64 and 246 isolated from raw meats. These strains gave a range of reactions in WH medium (3), WH medium modified by Microbiological Sciences, Inc. (1) (MS), and WH medium modified by us.

All media were prepared 1 day before the experiments were begun and stored in petri plates overnight in a refrigerator. *Clostridium welchii* type A antitoxin (0.06 ml) (Wellcome Laboratories) was spread on one-half of each plate, and the plates were dried (2). To prepare the inoculum, the cultures were incubated in cooked medium for 24 h and a loopful of culture was transferred to another tube of cooked medium. The second tube was incubated for 24 h, and the transfer and incubation procedures were repeated until the cultures had been incubated for four 24-h periods. A loopful of culture was transferred to each plate. The jars containing the plates were evacuated, filled with a mixture of 90% N_2 and 10% CO_2, and incubated for 24 h at 37.3 ± 0.5°C. After this incubation, the plates were divided into three groups, each with duplicate plates of the three media. One group was incubated anaerobically for several days at 37.3 ± 0.5°C, the second was incubated under anaerobic conditions at 31.3 ± 0.5°C, and the third was incubated at 5.8 ± 2.0°C. The plates were examined every 24 h for growth, color, and presence and enlargement of the lecithinase halo. Measurement of the lecithinase reaction was made with calipers, and the mean of three measurements at different times was plotted.

The information given in Fig. 1 for strain C and in Fig. 2 for strain III represent the range of results obtained. All four strains were positive in our modified medium (Fig. 3), whereas only two (strains III and 64) were positive in the MS and WH media, with a very weak reaction in the latter medium. Incubation anaerobically for more than 24 h is not necessary, since the reaction continues to increase when placed under

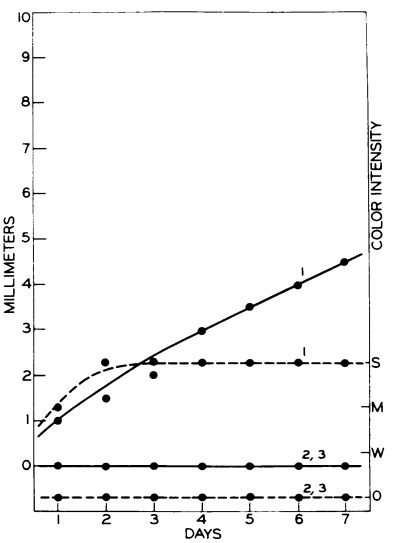

FIG. 1. *Zone size and color intensity of the lecithinase reaction under anaerobic incubation at 37.3 ± 0.5°C for culture C of C. perfringens. 1, Our modified medium; 2, MS medium; 3, WH medium. (——) Zone size; (-----) color intensity: S, strong; M, medium; W, weak.*

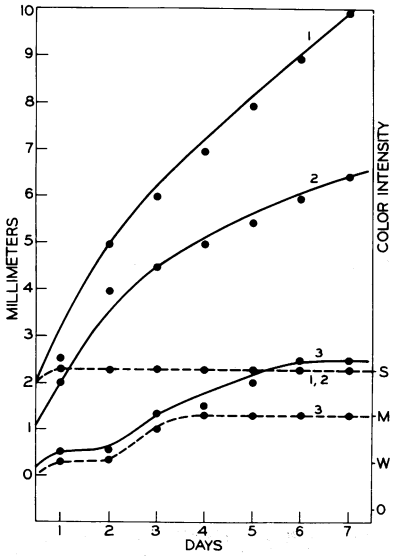

FIG. 2. *Zone size and color intensity of the lecithinase reaction under anaerobic incubation at 37.3 ± 0.5°C for 24 h followed by aerobic incubation at 31.3 ± 0.5°C for culture III of C. perfringens. 1, Our modified medium; 2, MS medium; 3, WH medium. (——) Zone size; (-----) color intensity: S, strong; M, medium; W, weak.*

aerobic conditions at 37°C. After 6 days the size of the halo in our modified medium increased, but not all of the strains showed this increase in the other two media (strain C, Fig. 1). Refrigeration apparently stopped the reaction, but the opalescent areas became more visible after holding at the lower temperatures. Some difficulties were experienced in growing the cultures in the WH medium, but they grew profusely in the other two media.

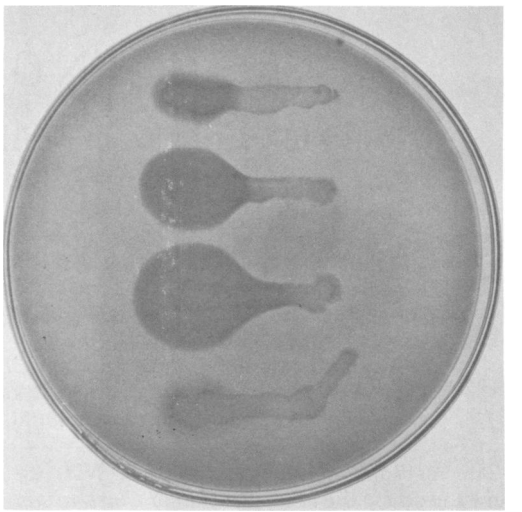

FIG. 3. *Zone size and color intensity of the lecithinase reaction in the medium modified by us after incubation under anaerobic conditions for 24 h. From top to bottom: cultures C, III, 64, and 246.*

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