Comparison of Schaedler Agar and Trypticase Soy-Yeast Extract Agar for the Cultivation of Anaerobic Bacteria

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Schaedler agar (SA) and Trypticase soy-yeast extract agar (TSYEA), both supplemented with rabbit blood (5%, v/v) and menadione (0.5 mg/liter), were compared with respect to quantitative recovery, quality of growth, and rapidity of growth of selected anaerobic bacteria. The media were stored for 2 to 4 days prior to use in an anaerobic glove box, where all subsequent bacteriological procedures were performed. After 24 hr of incubation, colonies of Clostridium cadaveris (C. capitovalve), C. haemolyticum, C. novyi A, and C. perfringens were larger on SA than on TSYEA, and the appearance of C. novyi B colonies on SA at 24 hr antedated their appearance on TSYEA. Quantitative recovery of C. novyi B was improved on SA; recovery of the other clostridia tested was comparable on the two media (inconclusive results were obtained with C. novyi A). Rough colonial types of some of the clostridia emerged on SA. No appreciable differences in results with the two media were noted for Bacteroides fragilis, B. melaninogenicus, or Fusobacterium fusiforme.

Some anaerobic bacteria are fastidious even when existing culture techniques are used, and isolation of such organisms from clinical specimens requires an inordinate amount of time. Recently, Mata et al. (4) reported that excellent growth of various anaerobes was obtained with a modification of the medium described by Schaedler et al. (6). We decided to compare the media used by Mata, available as Schaedler Agar (SA; BBL), with Trypticase-soy-yeast extract agar (TSYEA) [the medium currently used in our laboratory (2)] with respect to quantitative recovery, quality of growth, and rapidity of growth of selected anaerobes.

MATERIALS AND METHODS

Media. The composition of SA (BBL) in grams per liter is as follows: Trypticase soy broth, 10.0; Polypeptone peptone, 5.0; dextrose, 5.0; yeast extract, 5.0; Tris(hydroxymethyl)-aminomethane, 3.0; hemin, 0.01; L-cystine, 0.4; and agar (dried), 13.5. TSYEA consisted of Trypticase soy agar (BBL) plus 0.5% yeast extract (Difco). Both media were autoclaved in the same day. The pH of TSYEA was adjusted to 7.3 to 7.5 with 1 N NaOH; the pH of SA, unadjusted, varied from 7.6 to 7.8. Both media were autoclaved at 121 C for 15 min. Before plates were poured, 0.5 mg of menadione [2-methyl-napthoquinone] per liter and 50 ml of defibrinated rabbit blood per liter were added to both media (2), and 0.01 g of hemin per liter was added to TSYEA to give the same concentration present in SA. The media were dispensed into glass petri dishes (15 by 100 mm) with Brewer metal tops (BBL) that contained absorbent discs to prevent accumulation of excess moisture. As soon as the media solidified (within 30 min after pouring), the plates were put into an anaerobic glove box and kept under anaerobic conditions for 2 to 4 days before use.

Anaerobic glove box. A flexible plastic anaerobic glove box, designed by Rolf Freter of the University of Michigan, was used. The design and operation of this system have been described previously (1). An electric incubator (Chicago Surgical and Electric Co.) was placed within the chamber for incubation of cultures. A gas mixture containing 85% N2, 10% H2, and 5% CO2 was used; the O2 concentration within the chamber, monitored with a Lockwood and McLeod Trace Oxygen Analyzer (model GP), was maintained at less than 10 ppm.

Stock cultures. Stock cultures of the anaerobic bacteria employed in the experiments were all from the Collection of the Center for Disease Control Anaerobe Laboratories, and these were maintained in chopped meat dextrose (2) or in thioglycollate broth (BBL, 135C) as required. The identity of each culture was confirmed by determining the cultural and biochemical characteristics (2) and the metabolic end products in a peptone-yeast extract-glucose broth culture (3).

Procedures. All of the bacteriological procedures were performed within the anaerobic glove box. Tubes of thioglycollate broth (BBL, 135C) were inoculated with the microorganisms to be tested and were incubated at 37 C until generalized turbidity occurred (18
**TABLE 1. Colony size at 24 hr and quantitative recovery of selected anaerobic bacteria on Trypticase soy-yeast extract agar and Schaedler agar**

| Organism                  | Dilution | Trypticase soy-yeast extract | Schaedler |
|---------------------------|----------|-----------------------------|-----------|
|                           |          | No. of colonies<sup>a</sup> | Colonial diameter range (mm)<sup>b</sup> | No. of colonies<sup>a</sup> | Colonial diameter range (mm)<sup>b</sup> |
| *Clostridium cadaveris*   | $10^{-4}$| 35(1)                       | 1.0-2.0   | 56(1)                       | 1.0-3.0   |
|                           | $10^{-4}$| 42                          | 1.0-2.0   | 42                          | 1.5-2.5   |
| *C. haemolyticum*         | $10^{-6}$| 115(2)                      | m         | 113(1)                      | 1.5-5.0   |
|                           |          | 123                         | m         | 10                          | m         |
|                           | $10^{-5}$| 3                           | m         | 4                           | m         |
| *C. novyi A*              | $10^{-4}$| 203(2)                      | m         | 131(2)                      | m-2.5     |
|                           |          | 203                         | m         | 136                         | m-2.5     |
|                           | $10^{-5}$| 12                          | m         | 15                          | m-2.5     |
| *C. novyi B*              | $10^{-4}$| 47(3)                       | 0         | 282(1)                      | 0.0-1.5   |
|                           |          | 27                          | 0         | 20                          | 0         |
| *C. perfringens*          | $10^{-7}$| 83(1)                       | 2.0       | 71(1)                       | 3.5-4.0   |
|                           |          | 96                          | 1.0-2.0   | 104                          | 3.5       |
| *C. tertium*              | $10^{-6}$| 300(1)                      | 0.5-1.0   | 279(1)                      | 0.5-1.0   |
|                           |          | 36                          | 1.0       | 18                          | 1.0       |
| *Bacteroides fragilis*    | $10^{-6}$| 307(1)                      | 0.5-1.0   | 390(1)                      | 0.5-1.0   |
|                           |          | 280                         | 0.5-1.0   | 373                         | 0.5-1.0   |
| *B. melaninogenicus*      | $10^{-5}$| 236(3)                      | m         | 204(3)                      | m         |
|                           |          | 229                         | m         | 330                         | m         |
| *Fusobacterium fusiforme* | $10^{-5}$| 394(2)                      | m         | 303(2)                      | m         |
|                           |          | 395                         | m         | 303                         | m         |
|                           | $10^{-6}$| 44                          | m         | 43                          | m         |
|                           |          | 57                          | m         | 46                          | m         |

<sup>a</sup> Numbers in parentheses indicate duration of incubation (days) prior to colony count.
<sup>b</sup> m = Minute.
<sup>c</sup> Sample of 0.3 ml instead of the usual 0.2 ml.

The results of the recovery of the clostridia tested, *C. cadaveris* (C. capitovalae), *C. haemolyticum*, *C. perfringens*, and *C. tertium*, were comparable on the two media. The recovery of *C. novyi* B was greater on SA than on TSYEA; the results for *C. novyi* A were inconclusive.

In addition to these differences, the clostridia tested produced only smooth colonies on TSYEA, colonies, only hemolytic zones, on TSYEA (minute colonies appeared at 48 hr). On SA, the colonies of *C. novyi* B were 1.0 to 1.7 mm in diameter (Fig. 1). Colonies of most of the other clostridial species tested were larger on SA than TSYEA. Colonies of *C. novyi* A and *C. haemolyticum* were minute on TSYEA but considerably larger on SA. Only *C. tertium* colonies were identical in diameter on the two media.
two agars for anaerobic bacterial growth

TWO AGARS FOR ANAEROBIC BACTERIAL GROWTH

FIG. 2. C. cadaveris (C. capitovale) after 24 hr of incubation at 37 C showing rough and smooth colony types.

DISCUSSION

In our comparative study, SA proved to be superior to TSYEA for cultivation of some of the most fastidious anaerobes. The appearance of sizable colonies (greater than 1.0 mm in diameter) of C. novyi B and C. haemolyticum on SA within 24 hr and the increased recovery of C. novyi B on SA were noteworthy, because these organisms are difficult to cultivate (7). In our experiments, the effect(s) of atmospheric oxygen was essentially eliminated. Therefore, factors in the SA must have been responsible for the improved growth. Moore showed that L-cysteine is a required factor for luxuriant surface growth of C. novyi B (5). It is possible that the L-cystine in SA is reduced and maintained as L-cysteine within the anaerobic glove box.

SA did not offer distinct advantages over TSYEA for the other clostridia tested. The development of larger colonies on SA might facilitate isolation from mixed specimens, but the emergence of rough colony types could complicate identification. Quantitative recovery was, for the most part, comparable with the two media.

Results with SA and TSYEA were comparable for the nonsporeforming, gram-negative anaerobic bacilli tested.

Studies with additional genera and species of anaerobic bacteria are in progress.

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