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Bacteroides Species: Maintenance of Laboratory Strains

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A medium composed of blood agar base (40 g/liter), yeast extract (5 g/liter), and cysteine hydrochloride (0.05 g/liter), completely filling screw-cap tubes (13 by 100 mm), can keep *Bacteroides* species alive for at least 10 months without refrigeration.

The role of anaerobes in bacteriological processes has been stressed in recent years because simple methods for their cultivation have been developed by R. E. Hungate (4) and others (3). As these methods became adapted to clinical use (6), the importance of anaerobes in disease (7), and more specifically in human disease (2), has become obvious.

We were interested in the physiology and antigenicity of *Bacteroides* species (5), but encountered difficulties in maintaining stock cultures of these organisms. The recommended method for maintenance of anaerobic bacteria is storage at room temperature in chopped-meat broth lacking carbohydrate (3; W. E. C. Moor, personal communication). There are several problems associated with the use of chopped-meat broth. One of these is its preparation. To quote from the *Anaerobe Laboratory Manual* (3), "Use 500 g lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water, and NaOH and bring to boil ... skim fat off surface, and filter" etc. A second problem with chopped-meat broth for storage is the turbidity of the medium before inoculation. This turbidity necessitates Gram staining to determine whether growth has occurred. Gram staining increases the possibility of oxygen and microbial contamination of the culture. This problem may be artificial, because of our background and training, but we favor transparent agar medium for storage of cultures.

We began some time ago to search for a replacement for chopped-meat broth. The medium now used in our laboratory for storage of anaerobes is a modified blood agar base medium. It is composed of blood agar base (Difco), 40 g/liter; yeast extract (Difco), 5 g/liter; and cysteine hydrochloride, 0.05 g/liter. From the Difco *Manual* (1), the contents of blood agar base can be seen to be similar to those of chopped-meat medium (3). Yeast extract and cysteine were shown experimentally to improve growth on blood agar base medium. The addition of a normal carbon source such as glucose causes the pH of the medium to decrease during growth, and this decrease adversely affects the longevity of the cultures.

The medium is prereduced and anaerobically sterilized in screw-cap tubes (13 by 100 mm) and inoculated with a loop long enough to reach the bottom of the tube. The tube is capped, sealed, incubated at 37°C for 2 days, and then stored at room temperature. After 10 months, all of the cultures, including *Bacteroides fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. distasonis*, were viable.

To compare growth in modified blood agar base with NIH thioglycollate (Difco), a Spectronic 20 spectrophotometer was connected to a strip chart recorder and the whole mechanism was incubated so that the cuvette in the light beam was at 37°C. A small sample of modified blood agar base was dissolved in cold water, and the agar was filtered out with a Whatman no. 1 filter to produce a liquid medium. Both the regular agar medium and the liquid medium were sterilized, and the agar medium was cooled to 56°C. About 4 ml of the liquid medium was added to a sterile cuvette and frozen in a dry ice and acetone mixture. The agar medium was then added to completely fill the remainder of the tube, and the agar was allowed to solidify. These cuvettes were sealed to preserve anaerobiosis. This procedure produced a cuvette with liquid modified blood agar base on the bottom and solidified medium as a plug on top. A similar procedure was used to produce NIH thioglycollate cuvettes.

A clinical isolate of *B. fragilis* was inoculated into the two different media, and the growth was compared. The doubling time in NIH thioglycollate was 7.5 h, whereas in modified blood agar base the doubling time was 3 h.

In conclusion, modified blood agar base seems to be an easy and economical method for the growth and maintenance of stock cultures of *Bacteroides* species.

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