Autoclaved Liquid Medium for Propagation of *Treponema hyodysenteriae*

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Three liquid media that differ slightly in composition but not in the method of preparation were developed for the propagation of *Treponema hyodysenteriae* and *Treponema innocens*. The three media are unique in that all components are sterilized by autoclaving before use. These media supported better growth of *T. hyodysenteriae* than did previously used liquid media.

*Treponema hyodysenteriae* is the etiologic agent of swine dysentery (1) and the cause of serious economic loss to the swine industry (8). The lack of reliable, reproducible growth of *T. hyodysenteriae* at a high titer in a liquid medium has been a significant impediment to research on this organism. We developed media for the propagation of *T. hyodysenteriae* which support the growth of greater than 1 x 109 cells per ml. The preparation of these media is greatly simplified because they are totally autoclavable.

*Treponema innocens*, a nonpathogenic treponeme morphologically identical to *T. hyodysenteriae* (4), was included in the study to assure that the media being tested could also support the growth of this organism.

Pathogenic isolates (5) of *T. hyodysenteriae* B78, A-1, B234, B169, and B204 were provided by D. L. Harris. Field isolates of *T. hyodysenteriae* B8075, B6922, B7056, and B6929 from recent cases of swine dysentery were provided by I. T. Egan, Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames. The isolates of *T. innocens* (4, 5; D. J. Taylor, Ph.D. dissertation, University of Cambridge, Cambridge, England, 1972) used in this study (4/71, B256, and Puppy) were also provided by D. L. Harris. Isolate B204 grown in maintenance medium was used throughout unless otherwise indicated.

Modified Kinyon medium was prepared as previously described (6) except that a stir bar (10 by 3 mm) was added to each tube.

Modified Lemcke medium was prepared as previously described (6), with several modifications. Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) was prepared while exposed to the ambient atmosphere and was dispensed into anaerobe tubes containing stir bars before autoclaving. At the time of inoculation, 10% fetal bovine serum, 0.2% (wt/vol) sterile sodium bicarbonate, 0.05% (wt/vol) sterile cysteine hydrochloride monohydrate, and 0.5% (wt/vol) sterile glucose were added to the tubes under a flow of 10% CO2-N2.

Maintenance medium was prepared by mixing TSB, 0.25% (wt/vol) glucose, 1.0% (wt/vol) yeast extract, and 4.0% (vol/vol) salts solution in distilled water. The medium was prereduced and anaerobically sterilized (2) in Hungate anaerobe tubes fitted with septum stoppers (Bellco Glass, Inc., Vineland, N.J.) and containing stir bars.

Three totally autoclavable media were prepared with the following components in common: TSB, 0.5% (wt/vol) glucose, 0.2% (wt/vol) sodium bicarbonate, 0.05% (wt/vol) cysteine hydrochloride monohydrate, and 1.0% (wt/vol) yeast extract. In addition, each of the media contained two of the following three components: 2.0% fetal bovine serum (S), a 1.5% cholesterol solution (C) (7), and 5.0% pig feces extract (F); the media were designated FS, FC, and SC. The media were dispensed into Hungate anaerobe tubes fitted with septum stoppers and containing stir bars.

The FS, FC, and SC media were prepared by prereduced anaerobic sterilization (2) methods. The TSB, glucose, sodium bicarbonate, yeast extract, and pig feces extract were mixed in distilled water, and the pH was adjusted to 6.85. The mixture was boiled for 10 min and then cooled to room temperature with reduced 10% CO2-N2 bubbling in the mixture. Cysteine hydrochloride monohydrate, fetal bovine serum, or cholesterol solution or a combination thereof was added, and the medium was dispensed in 5-ml amounts into Hungate anaerobe tubes fitted with septum stoppers and containing stir bars under a flow of 10% CO2-N2. The medium was sterilized by autoclaving for 15 min at 121°C.

Pig feces extract was prepared with feces from a pig free of swine dysentery and receiving nonmedicated feed. One part feces was combined with four parts phosphate-buffered saline (0.01 M, pH 7.2 to 7.4) and mixed with a magnetic stir bar for 20 to 24 h at 4°C. The mixture was centrifuged at 10,000 x g for 1 h, and the supernatant was withdrawn and frozen at -80°C.

Modified Kinyon medium was inoculated as previously described (3). Modified Lemcke medium was inoculated under a flow of 10% CO2-N2. The FS, FC, and SC media were inoculated via a syringe and needle, with the needle penetrating the rubber septum stopper of the Hungate anaerobe tube. All broth cultures contained magnetic stir bars spinning at 500 rpm and were incubated upright at 38°C.

Inocula for determination of total cell counts and optical density were grown in maintenance medium (for 48 h at 38°C). The inoculum was 2% of the final volume and was approximately 105 organisms per 5 ml of medium. All cultures used in our studies were pure as determined by inoculation of aerobic blood agar plates and thioglycolate broth. Uninoculated tubes served as controls. Quantitation of growth was done by two methods: (i) determination of total cell numbers, after dilution of the culture in phosphate-

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CELL COUNT

(ii) counts with 50-h incubation observed after saline, and 0.5-ml counts.

Lemcke and modified Kinyon Quantitation and SC media (Fig. FC, less than satisfactory in to lower significantly x.

FIG. | TOTAL CELL COUNT

| F/C | F/S | S/C | Lemcke (modified) | Kinyone (modified) |

| 5x10^9 | 1x10^8 | 1x10^7 |

TOTAL CELL COUNT

INOCULATION TIME (Hours) 0 5 10 15 20 25 30 35 40 45 50

5x10^6 1x10^5

All of the T. hyodysenteriae isolates of three new liquid media. FS and FC failed to support the growth of one and three isolates of T. hyodysenteriae, respectively. All of the T. hyodysenteriae isolates grew in SC medium. All three media supported the growth of the T. innocens isolates.

T. hyodysenteriae B204 (initially passage 11 in vitro) was serially transferred at comparable intervals in FS, FC, and SC media; growth was successfully supported through six serial passages without indication of diminishing titers. Strain B204 was inoculated into FS, FC, and SC media which had been prepared 63 days earlier and stored at room temperature and into FS, FC, and SC media prepared on the day of inoculation. The organism grew equally well in both sets of media, as determined by optical density readings.

Four dysentery-free pigs approximately 2 months old and weighing 50 to 70 lbs (ca. 23 to 32 kg), housed in a small confinement unit and receiving nonmedicated pelleted feed, were challenged exposed via a stomach tube with 20 ml of T. hyodysenteriae grown in SC medium for 32 h at 38°C. The final cell count was 1 x 10^9/ml. Three dysentery-free control pigs of the same age and weight were housed in a separate pen within the unit and were not inoculated. Clinical signs of swine dysentery were observed in three of the four challenge-exposed pigs. T. hyodysenteriae was isolated from rectal swabs from all four challenge-exposed pigs. The three un inoculated control pigs did not develop clinical signs of swine dysentery nor was T. hyodysenteriae isolated.

Simplified and improved media for the propagation of T. hyodysenteriae and T. innocens were developed. The results of our studies show that three totally autoclavable media were capable of supporting excellent growth of T. hyodysenteriae and T. innocens. Growth, measured by total cell numbers and optical density, was similar or better than growth in media used in previous research. Continued use of the three media over a 2-year period resulted in consistent cell counts ranging from 1 x 10^9 to 4.5 x 10^9 organisms per ml in volumes ranging from 5 to 1,000 ml.

The unique aspect of the media is that all components are obtained before autolysis, eliminating the need to add additional components at the time of inoculation. The media are dispensed in Hungate anaerobe tubes fitted with septum stoppers under a flow of 10% CO_2 and N_2. Transfer of inocula is done easily with a syringe and needle by penetrating the stopper. The three media (FS, FC, and SC) differ in composition but not in the method of preparation, and each gave similar results. Our experience did not show one of the three media to be superior to the others. The availability or ease of preparation of the components may make one of the three more attractive to various laboratories.
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LITERATURE CITED


