Axenic culture of *Giardia lamblia* in TYI-S-33 medium supplemented with bile

DAVID B. KEISTER

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, USA

TYI-S-33 medium was developed for axenic culture of *Entamoeba histolytica* (DIAMOND *et al.* 1978). This medium, supplemented with bovine bile, can be used to isolate, axenize and culture *Giardia lamblia*.

G. lamblia trophozoites were first cultured axenically in HSP-1 medium by MEYER (1976). Subsequently, TP-S-1 medium, developed for culture of *E. histolytica* by DIAMOND (1968), was sterilized by filtration and used by VISVESVARA (1980) to adapt gradually and culture Meyer's original strain (Portland-1) with shortened generation time of about 12 hours and increased cell yields. GILLIN & DIAMOND (1981) obtained the Portland-1 strain from Visvesvara and cultured it in filter-sterilized TP-S-1 and TYI-S-33 media containing various concentrations of cysteine. Growth was marginal in TYI-S-33 medium in which the cysteine requirement tended to be very specific and substantially better in TP-S-1 medium, where the cysteine requirement could be partially satisfied by other sulphydryl compounds.

Because *G. lamblia* colonizes the proximal small intestine of its host and may establish in this location in response to the bile-rich environment, several preparations of crude bile and purified bile salts were added to TYI-S-33 medium to determine if they influenced the growth of the parasite. The addition of crude bile* improved growth so substantially, that it exceeded that achieved with TP-S-1 medium.

The modified version of TYI-S-33 medium used for the culture of *G. lamblia* contins bile and additional cysteine. Each 100 ml of medium contains the following: 100 mg K_2HPO_4, 60 mg KH_2PO_4, 2 g Trypticase, 1·0 g yeast extract, 1·0 g glucose, 200 mg NaCl, 200 mg cysteine-HCl monohydrate, 20 mg ascorbic acid, 2·28 mg ferric ammonium citrate, 50-100 mg dehydrated bovine bile and 10 ml of inactivated bovine serum (56°C, 30 min). The vitamin-Tween 80 mixture added to TYI-S-33 medium by DIAMOND *et al.* (1978) does not enhance the growth of *G. lamblia* in bile-supplemented medium and is omitted. The pH of the medium, before the addition of serum, is adjusted to 7·7·2 with 1 N NaOH. It is sterilized by passage through a 0·45 μm membrane filter, supplemented with serum and dispensed into tubes, flasks or bottles filled to at least 80% capacity. The medium can be stored for 7 to 10 days at 4°C without significant loss of effectiveness. Medium prepared without cysteine and ascorbic acid can be stored in the same manner for a much longer period of time, but requires the addition of a concentrated, filter-sterilized solution of neutralized cysteine and ascorbic acid just before use.

G. lamblia stocks are usually maintained in 13 ml of medium in borosilicate glass tubes (16 × 125 mm) sealed with tightly fitting rubber-lined screw caps. The culture tubes are incubated at 36°C in a horizontal position at a slight incline, and are subcultured at intervals of 72 and 96 hours. The cells form a dense, adherent monolayer on the surface of the glass and are dislodged by repeated gentle inversion of the culture tube after chilling for at least 10 min in an ice water bath. 10 to 100 μl of a heavy cell suspension (usually 2×10^4 to 2×10^5 cells) is sufficient to generate maximum growth during the subsequent three to four day cycle of growth. When necessary, cell concentrations are enumerated with a Coulter counter and the inoculum size is adjusted accordingly.

Four strains of *G. lamblia* were tested for growth in bile-supplemented TYI-S-33 medium. Portland-1 (ATCC #30888) was obtained from G. Visvesvara at the Centers for Disease Control in Atlanta, Georgia, USA. A second strain, WB (ATCC #30957), was isolated in bile supplemented TYI-S-33 medium by F. Gillin at the Natinal Institutes of Health in Bethesda, Maryland (SMITH *et al.*, 1982). Two additional strains, RS and LT which were isolated and maintained in TPS-1 medium were obtained from M. Wittner at the Albert Einstein College of Medicine in New York City.

Portland-1, RS and LT grew very well in bile-supplemented TYI-S-33 medium from the first subculture onward, with yields exceeding those obtained in TP-S-1 medium and with significant decreases in generation time. All three strains could be passed alternately in the two media, without an adaptive procedure, and with predictable changes in growth rate and yield. The WB strain which was isolated and maintained in bile-supplemented TYI-S-33 medium, would not grow in TP-S-1 medium or in unsupplemented TYI-S-33 medium until it had been continuously cultured in bile-supplemented TYI-S-33 medium for about nine months. All four strains showed some additional improvement in growth rate and yield after being maintained in this medium for a long time.

The adherent monolayer of cells in *G. lamblia* stock cultures (13 ml of medium) represents about 80-90% of the total cell population during the log phase of growth and about 60% or less in the stationary phase. Trophozoite densities approach or exceed 2×10^6 cells per ml and the minimum generation time is six to seven hours.

The cells can be cultured in tightly closed glass or

*One preparation of bile distributed by Sigma Chemical Company (B-8381) was particularly useful because different batches of this product were more reliable than any of the other bile products tested.

plastic tubes, flasks or bottles of varied shapes and size in a horizontal or vertical orientation. However, cell yields per unit volume of medium are reduced when the ratio of culture vessel surface to medium volume is decreased.

The usefulness of bile-supplemented TYI-S-33 medium for isolation of *G. lamblia* trophozoites from duodenal fluid of a giardiasis patient is based on only one direct attempt, which resulted in the establishment of the W.B. strain. However, all four strains have been cultured easily and re-axenized in this medium following isolation from the small intestine of 10 to 14-day-old Swiss mice, infected as neonates by percutaneous intragastric inoculation with 10^4 or 10^5 trophozoites (Keister & Mattern, manuscript in preparation).

References

Diamond, L. S. (1968). Techniques of axenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amoeba. *Journal of Parasitology*, **54**, 1047-1056.

Diamond, L. S., Harlow, D. R. & Cunnick, C. C. (1978). A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 431-432.

Gillin, F. D. & Diamond, L. S. (1981). *Entamoeba histolytica* and *Giardia lamblia*: Growth responses to reducing agents. *Experimental Parasitology*, **51**, 382-391.

Meyer, E. A. (1976). *Giardia lamblia*: Isolation and axenic cultivation. *Experimental Parasitology*, **39**, 101-105.

Smith, P. D., Gillin, F. D., Spira, W. M. & Nash, T. E. (1982). Chronic giardiasis: Studies on drug sensitivity, toxin production, and host immune response. *Gastroenterology*, **83**, 797-803.

Visvesvara, G. S. (1980). Axenic growth of *Giardia lamblia* in Diamond's TP-S-1 medium. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **74**, 213-215.

Accepted for publication 10th December, 1982.