Zeitschrift: Acta Tropica

Herausgeber: Schweizerisches Tropeninstitut (Basel)

Band: 36 (1979)

Heft: 3

Artikel: Cultivation and in vitro cloning of procyclic culture forms of

"Trypanosoma brucei" in a semi-defined medium : short communication

Autor: Schönenberger, M. / Brun, R.

DOI: https://doi.org/10.5169/seals-312533

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. Mehr erfahren

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. En savoir plus

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. Find out more

Download PDF: 25.11.2025

ETH-Bibliothek Zürich, E-Periodica, https://www.e-periodica.ch

Swiss Tropical Institute, Basel, Switzerland

Cultivation and in vitro cloning of procyclic culture forms of *Trypanosoma brucei* in a semi-defined medium*

Short communication

R. Brun, M. Schönenberger

Attempts to cultivate the vector forms of *Trypanosoma brucei* in defined media have made good progress in the last few years. Good growth of most *T. brucei* stocks which were tested could be achieved in semi-defined media (supplemented with 5–10% foetal bovine serum): RE I (Steiger and Steiger, 1976) and modifications of this medium (R. Steiger, personal communication); SDM-77 (Brun and Jenni, 1977); SM (Cunningham, 1977). Fully defined media (e.g. HX 25, Cross and Manning, 1973) are still unsuitable for most *T. brucei* stocks.

The medium SDM-77 has been used extensively in our laboratory and has been improved several times, resulting in the modification SDM-79 (Tab. 1). The main alterations are the following: lower concentration of glucose; higher concentrations of Na-pyruvate, Na-bicarbonate, adenosine and guanosine; addition of folic acid and a group of amino acids which are known to be present in tsetse fly haemolymph (Cunningham and Slater, 1974).

Several strains and stocks of *T. brucei* and *T. b. rhodesiense* were grown for several months in T-25 tissue culture flasks containing 5 ml of medium at 27° C, with two weekly subcultures. The trypanosome material was the same as used in an earlier investigation (Brun and Jenni, 1977). Additionally, the monomorphic derivatives of the *T. brucei* stocks of S427 and LUMP 227 were also grown.

The growth characteristics of *T. brucei* stock STIB 366 (a derivative of S42/030) is given in Fig. 1. In SDM-79 the maximum cell density after 4 days was about twice that in SDM-77. The stationary phase in SDM-79 (with 5% or 10% inactivated FBS) is less than 24 h; the high cell density after 4 days very rapidly makes the medium unsuitable for the growth and survival of the trypanosomes. With a lower serum concentration of 2.5% the trypanosomes remain longer in the stationary phase but the final cell density is lower than with higher serum concentrations.

* This study was supported by the Swiss National Science Foundation Grant 3.346-0.78

Correspondence: Dr. Reto Brun, Swiss Tropical Institute, Socinstrasse 57, CH-4051 Basel

Components per liter:

```
7.0 g MEM, F-14, powder (GIBCO Bio Cult)
```

- 2.0 g Medium 199 TC 45, powder (Wellcome)
- 8.0 ml MEM amino acids ($50 \times$), w/o L-glutamine (GIBCO Bio Cult)
- 6.0 ml MEM nonessential amino acids (100×) (GIBCO Bio Cult)
- 1.0 g glucose
- 8.0 g HEPES (= 33.6 mM)
- 5.0 g MOPS (= 24.0 mM)
- 2.0 g NaHCO₃
- 100 mg Na-pyruvate
- 200 mg L-alanine
- 100 mg L-arginine
- 300 mg L-glutamine
- 70 mg L-methionine
- 80 mg L-phenylalanine
- 600 mg L-proline
- 60 mg L-serine
- 160 mg L-taurine
- 350 mg L-threonine
- 100 mg L-tyrosine
- 10 mg adenosine
- 10 mg guanosine
- 50 mg glucosamine-HC1
- 4 mg folic acid
- 2 mg p-aminobenzoic acid
- 0.2 mg biotin

The pH is adjusted with 2N NaOH to 7.3 and the medium filter sterilized with a Millipore filter 0.22 μ m. Bovine haemin stock solution (2.5 mg/ml in 0.05 N NaOH) is autoclaved and added at a final concentration of 7.5 mg/l. Before use the medium is supplemented with 2.5 to 10% inactivated foetal bovine serum (GIBCO Bio Cult).

Tonicity of complete medium: 330 milliosmolar.

Two different trypanosome sources can be used to initiate a culture: 1. bloodstream forms from an infected mammalian host, 2. midgut forms from an infected *Glossina*. Bloodstream forms are the common source for all pleomorphic strains. Midgut forms from infected tsetse flies are often the only possibility to initiate a culture of monomorphic strains which hardly transform to culture forms in vitro.

To adapt bloodstream forms to culture conditions a parasitaemia with mainly intermediate and stumpy forms is needed (Brown et al., 1973; Ghiotto et al., in press). Citrated heart blood from a mouse or a rat was mixed with an equal volume of medium. After centrifugation for 7 min at 1000 g the supernatant was discarded and the pellet (trypanosomes and blood cells) resuspended in SDM-79 to a trypanosome concentration of about $5 \times 10^6 / \text{ml}$. The medium was supplemented with additional glucose (3 mg/ml) and penicillin/

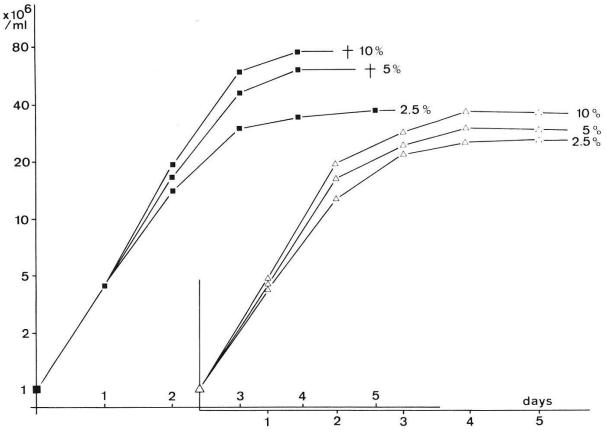


Fig. 1. Growth curves of established procyclic culture forms of *Trypanosoma brucei* STIB 366 at 27°C in SDM-79 (—————) and SDM-77 (——— Δ ——) supplemented with 2.5%, 5% and 10% inactivated foetal bovine serum. † = death of the culture.

streptomycin (250 U/ml, 250 μ g/ml respectively). After 24 h the medium was exchanged by centrifugation. After 48 h the blood cells were removed by slow centrifugation for 20 min at 50 g. The trypanosomes were pelleted from the supernatant (7 min at 1000 g) and resuspended in SDM-79 without additional glucose and with lower concentrations of antibiotics.

Whenever bloodstream forms cannot be adapted to in vitro conditions, midgut forms from infected tsetse flies are an excellent source for the initiation of a culture. An infected *Glossina* was killed and dissected under sterile conditions. The midgut was removed, opened and the gut forms transferred into SDM-79 with 500 U/ml penicillin and $500 \,\mu g/ml$ streptomycin for the first 48 h.

To find out the lowest number of trypanosomes required for subcultivation in SDM-79, different inocula ranging from $10^6/\text{ml}$ to $10^3/\text{ml}$ were checked. For *T. brucei* STIB 366 an inoculum of $10^3/\text{ml}$ was sufficient to start a new subculture. After 14 days the cell density in this culture reached $6 \times 10^7/\text{ml}$. Based on this finding, we tried to clone STIB 366 in vitro. For that purpose a good culture in logarithmic growth phase was diluted to approx. 10^4 trypanosomes per ml. All the peripheral wells of a Micro Test II tissue culture plate (Falcon) were filled with sterile distilled water to provide a humid atmosphere during the period of observation. With a drawn out capillary tube a tiny drop was placed at

the bottom of an empty well, the plate covered immediately and the drop examined in an inverted phase contrast microscope. If neither of two observers could detect more than one trypanosome, $100 \mu l$ of the following cloning medium was added to the well:

- 6.0 ml SDM-79 (with haemin and 10% inactivated FBS), 3–4 days conditioned by STIB 366 procyclic culture forms
- 6.0 ml SDM-79 (with haemin)
- 2.0 ml double distilled water
- 1.3 ml inactivated FBS
- -0.4 ml penicillin/streptomycin (100 \times)
- Filter sterilized with Millipore 0.22 μ m
- Tonicity: 290 milliosmolar.

The well was covered with Parafilm and the plate was sealed by a lid. After 7 days, $50 \,\mu$ l medium was carefully removed from the top of the well and $100 \,\mu$ l fresh cloning medium was added. After about 2 weeks or whenever the well showed a sufficient cell density, the whole well was transferred into a T-25 tissue culture flask with 3.0 ml medium. In one experiment on day 19 when the cell density reached $6 \times 10^7/\text{ml}$, the first subculture could be made.

Besides its excellent properties for the trypanosomes of the *T. brucei* subgroup, SDM-79 proved to be a very good culture medium for promastigotes of *Leishmania* species. *Leishmania donovani*. *L. tropica* and *L. braziliensis* were grown with two weekly subcultures for over one year in this medium revealing growth characteristics similar to *T. brucei*.

- 1 Brown R. C., Evans D. A., Vickerman K.: Changes in oxidative metabolism and ultrastructure accompanying differentiation of the mitochondrion in *Trypanosoma brucei*. Int. J. Parasit. *3*, 691–704 (1973).
- 2 Brun R., Jenni L.: A new semi-defined medium for *Trypanosoma brucei* sspp. Acta trop. (Basel) 34, 21–33 (1977).
- 3 Cross G. A. M., Manning J. C.: Cultivation of *Trypanosoma brucei* sspp. in semi-defined and defined media. Parasitology 67, 315–331 (1973).
- 4 Cunningham I., Slater J. S.: Amino acid analysis of haemolymph of *Glossina morsitans morsitans* (Westwood). Acta trop. (Basel) 31, 83–88 (1974).
- 5 Cunningham I.: New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. 24, 325–329 (1977).
- 6 Ghiotto V., Brun R., Jenni L., Hecker H.: *Trypanosoma brucei*: Morphological changes and loss of infectivity during transformation of bloodstream forms to procyclic culture forms. Exp. Parasit. (in press).
- 7 Steiger R. F., Steiger E.: A defined medium for cultivating *Leishmania donovani* and *L. braziliensis*. J. Parasit. 62, 1010–1011 (1976).