A culture method for study of the cell system in *Entamoeba invadens*.

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Undoubtedly, axenic methods are elegant culturing procedure and the absence of contaminants is good for biochemical investigations. How good however for studying the cell system in *Entamoeba*ae remains questionable on account of the absence of certain features of the natural habitat (e.g. food-bacteria, bacterial metabolites, redox potentials determined by bacteria). For this reason we returned to the bacterial association and developed a culture medium able to support amoebic growth by inhibiting bacterial propagation.

The basic medium

Some weeks before utilization a 10% beef serum solution was autoclaved. A beneficial sedimentation occurred at room temperature. The supernatant was diluted to a basic medium with sodium monophosphate-sodium diphosphate buffer (pH 6.0, 1200mOs/m/l=0.4M) and distilled water, both previously autoclaved. Dilution was made in a 5:2:3 ratio.

Preconditioning

Preconditioning occurred at 37°C with *Serratia marcescens*. Incubation was stopped 48 hrs. later and bacteria removed by centrifugation at 5000 rpm.

Enrichment

An enrichment supplement was freshly prepared by mixing beef serum with 22% meat extract and 60% glucose (both autoclaved stock solutions). The ratio was 50:15:5.
An antibiotic mixture containing 1 g. Streptomycin and 25 mg. Erycinum, dissolved in 6 ml. water, was added to 70 ml. enrichment supplement. Finally, the supplement containing antibiotics was added to the preconditioned medium in the ratio 1:3. This complex medium was then used for amoebic growth.

**Refrigeration**
The medium could be kept for a longer time at -20°C. Gentle thawing at room temperature was required to avoid precipitation.

**Bacteria for feeding**
_Aerobacter aerogenes_ was successfully used as particulate food. Since inhibited by streptomycin and erycinum, it remained metabolically active during amoebic culturing. Experience showed that bacteria killed by heating did not support amoebic growth.

In order to yield bacteria able to induce phagocytosis, _Aerobacter_ was grown at 37°C in a medium containing beef serum. The same basic medium described above was used after enrichment with Bacto Nutrient Broth (concentration of BNB 2%). Bacteria grown in pure BNB were not ingested by amoebae. Logarithmically grown bacteria were centrifuged and collected in phosphate buffer pH 6.0, 250 mOsm/l. Cells kept for several days at 4°C could also be used.

**Amoebic growth**
Amoebae and bacteria were inoculated together and centrifuged immediately at the bottom of the culture vessel. Cultivation occurred at 28°C. Amoebae removed bacteria from sediment. The medium remained permanently transparent. Superinfections producing precipitation were therefore easy to detect. Not more than 10⁻⁴ g. bacteria/ml. culture medium could be employed. At higher concentration undesirable opacity occurred.

We used: *Entamoeba invadens* (strain TR 2 nr.1), *Aerobacter aerogenes* (strain AH₈) and *Serratia marcescens* from the Hygiene Institute Tübingen, beef serum (ORKP 44/45 from Behringwerke, Marburg), meat extract (E. Merck, Darmstadt) Erycinum (Schering, Berlin).

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Dear Dr. Mr. M. H. S. C.,

Many thanks for your letter of 14.1./1974 and the submission of the title of your (and your colleagues) very interesting short communication in section 211 and 53.

May I expect, as far as it is possible on your side, the abstract of your presenting paper - being not longer than 300 words - already during the month of February (deadline is 30th March).

Submission of abstract:

(Section)

>Title

Surname, first name, address

Text of the abstract (not more than 300 words)

I hope that you have received at the end of December 1973 the third announcement with further informations.

With best regards,

Yours sincerely

[Signature]

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