A simple method for obtaining protozoa in pure culture *

by

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(Received for publication September 30, 1960)

It is now generally recognized that most types of physiological work on the protozoa are greatly restricted in their scope unless the organism under investigation can be raised in pure culture, that is, in the absence of any other organisms. This is particularly true in the field of biochemical investigations, where the presence of other living organisms usually vitiate the results. This fact has led many investigators to try to obtain new species of protozoa in pure culture. Of those which have unquestionably been made to grow in pure culture, between three and seven species[1] of Ciliates, hace belonged to what FURGASON (4) has called the *"Glaucoma-Colpidium* group", and Corliss (2, 3) has called the *"Colpidium-Glaucoma-Leucophrys-Tetrahymena* group". *Paramecium* is the only Ciliate not belonging to this group that has been achieved in pure culture (6, 11).

It is possible that many other of the common Ciliates may be made to grow in pure culture, but the amount of effort required to isolate all of the species found in a given sample taken from nature makes the determination of their potentiality impractical by conventional methods.

Different degrees of success have been attributed to methods employing antibiotics for obtaining protozoa in pure culture. ADLER and PULVERTAFT (1) used penicillin to help to purify a culture of the Flagellate parasite *Trichomonas vaginalis*. Subsequently the use of penicillin was extended to Ciliates by SEAMAN (10). PHELPS (9) described briefly a method for using streptomycin as an aid in purifying protozoa obtained from nature.

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* This work was made possible by funds from The Research Institute of The University of Texas, and was carried out at the Department of Zoology, The University of Texas, Austin, Texas.

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1 The precise number of species in this group which have been obtained in pure culture is questionable since taxonomists are currently at variance on this point.

The objetive of the present work was to develop a simple and effective method for discovering and isolating in pure culture all of the Ciliates in a water sample which can be made to grow in a particular nutrient medium.

MATERIALS AND METHODS

The method which has been developed for archieving this objetive consists of two parts: a) the enrichment of a water sample with a nutrient material to which has been added antibiotics to inhibit or delay the growth of most contaminants; b) the final purification of the culture by permitting the protozoa in the sample to swim through a migration tube.

a) We have tested the following antibiotic drugs in varying concentrations and combinations: penicillin, streptomycin, chloramphenicol (chloromycetin), terramycin, aureomycin, bacitracin, tyrothricin, and several of the sulfa drugs which were added to the natural water sample together with Bacto-Proteose Peptone (0.5 per cent). It was found that the most effective combination to inhibit growth of the bacterial flora, and still permit growth of protozoa consisted of approximately 0.5 mg/ml of streptomycin and 0.1 mg/ml of sulfathiazole. This combination has been observed to permit growth of the commonly ocurring Ciliates in contaminated cultures and in no way does it inhibit the rapid growth of members of the "*Colpidium-Glaucoma-Leucophrys-Tetrahymena* group" in pure culture. Bacterial growth is arrested in water samples exposed to this treatment in about half of the cases. Various concentrations of "Moldex" (Turtox) and of trichothecin have been tried in an attempt to inhibit the growth of molds, but without success; we have found that concentrations just sufficient to discourage molds are also toxic to protozoa. This preliminary treatment of the samples does not render the protozoa strictly bacteria-free but allows them to grow into visible populations so that a simple inspection will determine which samples may contain forms capable of surviving in pure culture and which should be rejected at this point. In general, good returns have been obtained by collecting between five and ten 10-ml samples from a given source.

(b) Since protozoa which have been subjected to the above treatment are rarely entirely free of contaminants, they are next subjected to treatment in a migration tube designed to effect complete purification. Migration tubes, which depend for their effectiveness on the principle that actively motile protozoa swim much more rapidly than their accompanying contaminants, and hence ultimately leave them behind, have been in occasional use. Most of them, however, have been so complicated or cumbersome as to limit their general use. The migration system which we have designed is shown in Figure 1, and consists of a piece of glass tubing about 120 cm long, 6 mm OD, and 4 mm ID, bent into a series of vertical convolusions. To one end of this tube a shortstemmed thistle tube is attached by means of a short piece of rubber tubing; the other end is attached by rubber tubing to a U-shaped piece of glass tubing, each arm being about 12 cm long. One arm of the U-tube passes through a two-

hole rubber stopper to the bottom of a 125 ml Erlenmeyer flask. A short bent piece of glass tubing is inserted in the other hole of the stopper and is plugged with cotton to maintain sterility in the flask. About 90 ml of medium, consisting of 0.5 per cent proteose peptone in tap water, is placed in the Erlenmeyer flask, a clamp is placed on the rubber tube to prevent the medium from flowing through the migration tube during autoclaving, and the mouth of the thistle tube is plugged with cotton. The neck of the Erlenmeyer flask and its rubber stopper are wrapped in cotton. The migration system mounted to a support-stand by two clamps and cushioned with a two-hole rubber stopper cut in halves in then autoclaved. If the system is to be used soon after autoclaving, the flask is shaken thoroughly to aerate the medium. The clamp is removed from the rubber tube and air is blown through the piece of glass tubing provided with sterile cotton in order to fill the system with medium. If bubbles of air remain in the system, they must be removed by sucking the medium back into the flask, and again forcing it through the glass tubing until the fluid in the cup and that in the flask are at the same level. A pinch clamp is applied to the rubber tubing and about 2 ml of medium is withdrawn from the thistle tube, 0.5 ml of the culture to be purified is added and the pinch clamp is removed from the rubber tube. This procedure insures against gravity flow of the contaminants in the direction of the flask. The set-up should not be disturbed until a visible population of protozoa is observed in the Erlenmeyer flask. In our experience, cultures usually go through the tube and form a population in the Erlenmeyer flask in about three days, but a few strains have taken as long as a week. After a population is observed in the flask, the pinch clamp is reapplied to the rubber tube, and the flask, U-tube, and rubber tube are removed from the rest of the system. The cotton wrapping the neck of the flask is removed, the flask is unstoppered, and samples are transferred to sterile media.

RESULTS

We have used the migration tube for the last ten years with an efficiency of between 90 and 95 per cent. Thirteen new strains of *Tetrahymena pyriformis* have been obtained by using the methods described in this paper. The origin of five of these, EZ, HS, LR, N, and Y, has been described (2). In addition, we have obtained BF and CR from San José, Costa Rica; AB from Abilene, Texas; PE from Ft. Stockton, N.M.; LA from Grants, N.M.; TC from Truth or Consequences, N.M.; and PR from Lake Florida, near San Juan, Puerto Rico.

Marked strain differences appeared within the species. Thus, strains TC, Y, and HS are thermophilic and grow at a temperature as high as 40.5°C, whereas ordinary strains of *T. pyriformis* will not grow at temperatures above 32° C. Strain N is partially thermophilic and will grow at a temperature as high as 37° C. Strains AB, LA, and PE produce a dark brown water soluble pigment during the first few months of growth in the laboratory but subse-

quently lose this potentiality. Biochemical differences have been observed among these stocks, the details of which are beyond the scope of this paper.

For routine purification of contaminated laboratory stock cultures it is not necessary to treat the culture of protozoa with antibiotics prior to putting it through the migration apparatus; nor is this pretreatment absolutely essential in attempting their original isolation from natural waters. Nevertheless it must be emphasized that in the latter case this step is quite useful in enhancing the likelihood of obtaining new forms from nature capable of growing in pure culture.

DISCUSSION

The conventional methods used to isolate protozoa in pure culture have depended upon the isolation of a single cell (5, 7, 8). In addition to being time consuming, this method permits the operator to wash only a few individual protozoan cells at a time. The method which has just been described requires little time and effort, and should afford an opportunity to isolate all protozoa present in a sample of natural water which are capable of living in pure culture in proteose peptone or other media. Actively motile protozoa will tend to swim throuh such a medium by random movement at a rate which exceeds by far the motility of accompanying bacteria; and if the protozoa can live and reproduce, they should migrate through the system sufficiently fast to leave all the bacteria behind unless their progress is blocked by some tropism. Their forward motion through the migration tube is probably encouraged by the fact that their environment at the starting point of the migration apparatus becomes unfavorable due to the exhaustion of oxygen and to the accumulation of excretory products.

It is particularly noteworthy that in every sample obtained from nature which yielded protozoa capable of living in axenic culture on proteose peptone, no protozoa were found other than those belonging to the "*C-G-L-T* group", and in every case these protozoa appeared to be *Tetrahymena*. It is very likely that using the appropiate medium other Ciliates may be obtained in pure culture employing the method just described.

SUMMARY

1. A simple and effective method is described for discovering and isolating in pure culture all of the Ciliates in a sample of natural water which can be made to grow axenically in a particular medium, making use of a combination of antibiotics and a migration system.

2. Construction and operation details of the migration apparatus are given.

3. Using proteose peptone as the growth medium, only members of the genus *Tetrahymena* were obtained from natural waters.

RESUMEN

1. Se describe un método sencillo y efectivo, en el que se emplea una combinación de antibióticos y un aparato de migración, para descubrir y aislar en cultivo puro todos aquellos ciliados presentes en una muestra de agua natural que sean capaces de crecer axénicamente en un medio dado.

2. Se dan los detalles para construir y operar el aparato de migración.

3. Empleando proteosa peptona como medio de cultivo, sólo se obtuvieron miembros del género *Tetrahymena* a partir de muestras de aguas naturales.

ZUSAMMENFASSUNG

1. Man beschreibt ein erfolgreiches und einfaches Vorgehen zur Isolierung in reine Kultur der in einer Süsswasserprobe vorhandenen Wimpertiere, die befähigt sind, auf einer bestimmten Nährlösung bakterienfrei zu gedeihen. Das Verfahren besteht aus einem Wanderungssystem und Anwendung von antibiotischen Mitteln.

2. Es wird die Anweisung zur Herstellung und Handhabung des Wanderungsapparates gegeben.

3. Bei Anwendung von Proteose-Peptone-Lösung als Nährboden, wurden bisher nur Vertretter der Gattung *Tetrahymena* aus Proben verschiedener Süsswassersammlungen isoliert.

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Fig. 1: Diagram of migration apparatus (explanation in text).