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Short Communication

Media and method for cultivation of soil ciliates

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Abstract

Two media - 'agricultural medium' and autoclaved sewage - were used to cultivate protozoa, particularly ciliates, from soil. 'Agricultural medium', was the refluxed extract of dried straw, leaf, groundnut cake, cowdung, ammonium sulphate and superphosphate. The method consisted of shaking 1% (wt/vol) soil samples in the media and counting the protozoa that developed at the end of aeration for 3,4 and 5 days.

Key words: Soil ciliates, 'agricultural medium', autoclaved sewage.

1. Introduction

Stout *et al*[1] reviewed the methods and recommended certain procedures for cultivation of protozoa from soil. They questioned: "Are flagellates and amoebae the most numerous MPN (most probable number) protozoa simply because they (flagellates and amoebae) are less susceptible to dilution shock and more easily fed than ciliates?". Since we carried out a fairly prolonged investigation on soil ciliates[2-7], we present here some of the results of our investigation on cultivation of ciliates from soil.

2. Materials and methods

2.1 'Agricultural medium'

This medium, which we developed, consisted of the following materials in one litre of distilled water.

Straw powder*	3.5 g
Leaf powder**	3.5 g
Cowdung***	3.5 g
Defatted groundnut cake	1.0 g
Ammonium sulphate	100 mg
Superphosphate	50 mg

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† Deceased

* Oven-dried rice straw milled and passed through 100-mesh sieve.

** Dried leaves of *Tabebuia* sp. (tree) on the campus of the Indian Institute of Science, Bangalore, were further dried in an oven (105°C), milled and passed through 100-mesh sieve. Analysis was not carried out to know if the straw (rice) or leaves (*Tabebuia* sp.) contained any pesticide residues.

*** Cowdung was first air dried and later dried in an oven (105°C) before passing the dried solids through 100-mesh sieve. Dried straw, leaves and cowdung that passed through 100-mesh were used to prepare the medium.

All the different materials used for preparing 'agricultural medium' are common materials that find their way either naturally or by agricultural practices to soil for crop production. The weighed constituents of the medium were added to 1 litre of distilled water and the contents were refluxed for ½ hour. The contents of the flask were cooled to room temperature (~25°C) and were filtered through Whatman No. 1 filter paper. The brown filtrate was autoclaved (121°C, 15 lb pressure) for ½ hour. The medium was cooled and stored in refrigerator for subsequent use.

2.2 Autoclaved sewage

The idea of using autoclaved sewage as a medium to cultivate soil protozoa was based on our observations that a variety of protozoa, especially ciliates, naturally developed in sewage under aerobic conditions. Sewage is rich in carbon, nitrogen, phosphorus, and also contains other growth promoting substances, including trace elements. Also, studies on the protozoa in sewage have shown that the ciliate protozoa have a definite role in the oxidative changes in the medium[8,9]. Further, sewage and soil have been viewed as similar systems[2,7, 10–14].

Effluent from a primary sewage settling tank was obtained from the sewage works on the campus of the Indian Institute of Science, Bangalore. The sewage was domestic in character and composition.

2.3 Method of cultivation

Samples of soil (1g) were added to the media (100 ml of medium–'agricultural medium' or autoclaved sewage) in 250 ml Erlenmeyer flasks. The flasks were shaken on a laboratory rotary shaker (200 rpm) at 25°C ± 2°C.

After shaking for 24/48/72/96/120 hours the flasks were placed on the laboratory bench and the contents were allowed to settle for ½ hour. Three replicate samples (0.05 ml) of the sediment or deposit were allowed and examined under a microscope (×150 magnification) for live (motile) ciliates. The number of different genera and species of ciliates and other protozoa were recorded and the average values from three replicates were calculated.

None of the earlier investigators reported results from 'shake-culture'.

3. Results and discussion

In this communication, typical numbers of soil ciliates, that developed in the two media tested, are given (Tables I and II).

The 'agricultural medium' (Table I) supported growth and activity of soil protozoa, especially ciliates. The number of ciliates is greater than those reported in the literature. This could be because of inherent ingredients of the medium, and/or the selective stimulation of development of aerobic ciliate protozoa through continuous aeration of the medium.

Table I

Soil ciliate protozoa that developed in 'agricultural medium'

(Counts $10^2/g^{-1}$ soil)

Soil samples (top soil, 15 cm) from different locations of Karnataka State, India	Ciliates that developed at the end of aeration for 72 hours	96 hours	120 hours
1	80	1370	620
2	80	1250	1100
3	Nil	80	420
4	950	370	160
5	400	1400	1300
6	450	150	40
7	Nil	Nil	40
8	650	1700	800
9	60	800	1100
10	1400	700	250
11	40	40	40

Generally, at the end of aeration even up to 48 hours, active ciliates were not detected as seen under the microscope Ciliates found were the species of *Chilophrya, Colpidium, Colpoda, Gonostomum, Lionotus, Stylonychia,* and *Vorticella.* Numerically, species of *Colpidium* and *Colpoda* were more. In addition to the ciliates, *Naegleria, gruberi* and *Hartmanella* sp. (Rhizopoda) and *Oikomonas termo* and *Cercomonas* sp. (Flagellata) also developed in the medium.

The results in Table II showed that, as in the case of the 'agricultural medium', autoclaved sewage supported the growth and activity of soil ciliates. However, general applicability or acceptability of autoclaved sewage for cultivation of soil ciliates may be debated on at least two counts. Firstly, availability of sewage, free from industrial wastes, on a routine basis for use as a medium could be a problem. Secondly, there is always a reservation as to whether the autoclaved sewage was completely free from viable cysts of protozoa. Even if a few cysts were resistant to autoclaving, such cysts could develop and grow in the medium, along with those from the inoculated sample of the soil, yielding higher numbers of protozoa for the soil tested. These two problems, do not allow autoclaved sewage as a first choice to cultivate soil ciliates.

The results (Tables I and II) showed that the numbers were not the highest at any particular time interval during the extended period of aeration (5 days). As some of the cysts could have remained dormant for longer periods than the others, examination and enumeration of numbers of ciliates were done at different intervals. At the same time, the proposed new method and media undoubtedly reduced the long periods of incubation time (10 to 15 days or longer) recommended by earlier investigators[15–17]. Reduction in incubation time was possible because of aeration of the medium which reduced some of the limitation to the natural process of development in comparison with static conditions of enumeration employed by the other investigators.

Table II

Soil ciliate protozoa that developed in autoclaved sewage

(Counts $\times 10^2/g^{-1}$ soil)

Soil samples (top soil, 15 cm) from different locations of Karnataka State, India	Ciliates that developed at the end of aeration for 72 hours	96 hours	120 hours
1	1200	320	200
2	900	240	900
3	60	120	780
4	1300	1300	60
5	1250	470	520
6	1000	1500	900
7	100	300	550
8	Nil	Nil	200
9	500	1600	1250
10	900	400	300
11	80	180	300

Generally, at the end of aeration even up to 48 hours, active ciliates were not detected as seen under the microscope.

Ciliates found were the species of *Chilophrya, Colpidium, Colpoda, Gonostomum, Linotus, Stylonychia,* and *Vorticella.* Numerically, species of *Colpidium* and *Colpoda* were more.

In addition to the ciliates, *Naegleria gruberi* and *Hartmanella* sp. (Rhizopoda) and *Oikomonas termo* and *Cercomonas* sp. (Flagellata) also developed in the medium.

All the methods of enumeration of microorganisms form soil basically depend on 'dilution technique' and the numbers reported represent, therefore, at the best MPN, and should not be taken as 'real' or absolute. However, if one were to evaluate the efficacy of a particular treatment to soil, or follow the progress of decomposition of organic matter, comparative numbers under identical conditions of growth and development, could prove useful. It was in that context that we found the 'agricultural medium' useful for investigations on soil protozoa.

There is a fundamental difference in the two media. In the 'agricultural medium', except for cowdung, other forms of organic matter (straw, leaf and groundnut cake) were in an undecomposed or raw state compared to autoclave sewage which was already in a decomposed state. This difference could also have influenced the growth and development of bacteria that were the basis of nutrition for ciliate protozoa.

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