The Axenixing of *Vorticella monilata*

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Sterile animals were needed for extension of experiments on the motility of vorticella[1] to include extraction of the contractile substance in the stalks; otherwise the nature of the extracts would be obscured with foreign contaminants. Therefore, the procedures described here was developed for removing its microbial associates with the help of antibiotics.

MATERIALS AND METHODS

The vorticellas used were clones isolated from populations taken out of local ponds and identified as *Vorticella monilata* TATEL according to Noland and Finley[2]. They were cultured for about two months on an egg yolk lettuce extract (EL) prepared as follows :

1. An extract of desiccated lettuce leaves (0.1 % , w/v) was made by boiling 5 min. in distilled water, and filtering.
2. A suspension of coagulated egg yolks (0.01 % . w/v) in distilled water was boiled 10 min. and filtered.
3. Equal parts of each solution were mixed before inoculation.

Cultures were refreshed every week with new EL and subcultured when necessary. EL was consistently successful in supporting luxurious populations of vorticellas.

Culture medium and vorticellas both contained in Petri dishes. Some small glass rods (1 cm x 0.5mm .) were also included in the culture dishes. Vorticellas attached to these, thus providing a " handle " by which some could conveniently be transferred for subculturing and other manipulations. When necessary, individuals were detached by gently prodding at the point of their attachment to the substratum with the tip of a micropipette.

All operations were performed under a hood (4 x 2 ft) which consisted of a glass top, plywood bottom, back, and sides, and a plastic drape over the front. A dissecting microscope whose oculars protruded through part of the drape was housed at one end. The floor of the hood was routinely covered with a sterile black sateen cloth before each experiment.

Transfer pipettes were drawn from soft-glass tubing to 0.3-0.8 mm diameter and plugged with cotton.

All glassware and other items were wrapped and autoclaved for 15 min. at 121°C .

Sterility was tested by inoculating treated vorticellas into tubes of nutrient broth and incubating at 37 °C . Controls were tubes which contained untreated animals and

uninoculated tubes.

RESULTS

Nutrient-agar streaks of EL within which vorticellas flourished revealed Gram-negative, motile, spore-forming rods as the major bacterial contaminants. These bacteria displayed high sensitivity towards achromycin and polymyxin by the conventional disc test.

These antibiotics were therefore tried to rid vorticellas of their bacterial associates. However, vorticellas tolerated achromycin (tetracycline HCL, crystalline, Lederle) at 5 mg/ml. in chalkley's fluid for at most 2 hr. After this interval, each coiled its stalk tightly and detached itself therefrom." Decapited " vorticellas contaminated the nutrient broth as did the glass rod to which they were attached. Similar results were evident with polymyxin B sulfate (Pfizer). Vorticellas were killed by this antibiotic when exposed for 1 hr. to 50 units/ml. of chalkley's fluid. The extreme toxicity of these antibiotics make further experimentation with them futile, as animals had to be left in sterile media for at least 5 hrs. for egestion of spores [3]. Therefore, a more compatible antibiotic was sought. Mixtures of ampicillin (as sodium salt, Beecham) plus streptomycin sulfate (Squibb) were prepared and glass rods with attached vorticellas were passed through a succession of washes interrupted by a stay in one of them for several hours. Some glass rods were then tested for contamination by placing in nutrient broth; others were tested for viability by dropping in EL.

When glass rods were passed through 5 washes of 2.5 mg ampicillin plus 250 μg. streptomycin/ml. in sterile EL, left 9.5 hr. in the 6th wash and then transferred through 5 more such baths, contamination was still evident. But now the major contaminants were yeasts which seemed to flourish in the relative absence of bacteria. In fact, surviving vorticellas became established in viability-test cultures. The bottoms of culture dishes were white with accumulated yeast cells, except around the bases of some vorticellas where there was a clear ring, visible to the unaided eye. This clear area was taken as evidence of active feeding upon yeast which supported the population for a limited time.

Examination by high powered microscope of the glass rods to which vorticellas were attached showed an occational yeast cell and some bacteria adhering in the slime which coated the glass; bacteria were also found sticking to various regions of the pellicle, and yeasts were held in clumps at the base of the stalks. This contamination could not be dislodged mechanically or chemically; e. g., adhering contaminants on vorticellas and rod resisted passage through 6 vertical columns of sterile EL (total — 300 c.c.) even after the viscosity was adjusted with agar to lower the rate of fall. Benzylammonium chloride (Roccal) was applied without success. This compound was extremely toxic: concentrations of 8 ppm. were lethal in 20 minutes. Similarly, vorticellas were killed by mystatin (Mycostatin, Squibb) after 2 hours in 50 units/ml. sterile EL.

Therefore use of vorticellas attached to glass rods was abandoned; later experiments were directed towards bacterial decontamination by streptomycin plus ampicillin and control over yeasts by dilution methods.

Ten individuals were detached from the substration captured in a sterile pipette, then transferred to a 1 ml. EL wash containing 2.5 mg ampicillin plus 250 μg streptomycin sulfate. Three such transfers were made. The 4th wash contained a 1 : 5 dilution of

the ampicillin + streptomycin used in the former baths. The vorticellas were left in this for 8 hrs., during which time they swam freely, being propelled by their rotating adoral membrance. They were then put through 2 washes, each again containing 2.5 mg ampicillin+ 250 μg streptomycin. Following this, all seven remaining animals were picked up in a sterile drop of EL and inoculated into nutrient broth. No contamination was evident in the broth even after incubation for as long as 40 days. This procedure has been repeated with as many as 30 animals.

Other methods of concentrating and washing have been attempted. Centrifugation was not too satisfactory as yeasts were carried down with the vorticellas even at the slowest speeds in the centrifuge. Furthermore, repeated washing by this means led to large losses of animals.

DISCUSSION

Most procedures for obtaining sterile, free-living ciliates embody one modification or another of the principles established by Parpart[3]. Parpart axenized paramecia by means of a series of rapid washes of single animals, interrupted by one lasting several hours for egestion of spores and their subsequent removal by dilution during further washes. The importance of swimming as a means of dislodging contaminants caught in cilia and cell surface irregularities was emphasized by Hetherington[5].

Others exploited trophic responses to increase the efficiency of the washing and dilution process[6,7,8,] or, more simply, centrifugation[9].

Efficiency of the washing and dilution procedures has been enhanced by the use of antibiotic[11]. Seaman[10] obtained sterile *Colpidium campylum* with relative few centrifugations using penicillin. Elliott[4] sterilized *Tetrahymena* with penicillin + streptomycin after a minimum of pipette transfers.

In this study, ampicillin + streptomycin were successful only when applied to detached and swimming animals. Detached animal were necessary because of the difficulty of controlling contaminants which stuck to their bodies and stalks, and the glass to which they adhered. The association of bacteria and some species of *Vorticella* was noted and figured by Noland and Finley[1]. Perhaps yeasts and bacteria were washed away from detached vorticellas as they swam to and fro to be diluted beyond consequence in the series of baths. Presumably, bacteria were also controlled by long term exposure to antibiotics.

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SUMMARY

Vorticellas were subjected to achromycin, polymxin, and mixtures of ampicillin-streptomycin to remove bacterial contaminants. Although achromycin and polymyxin were most effective in controlling bacteria isolated from vorticella cultures, but they proved too toxic for practical use. However, certain dilutions of ampicillin-streptomycin were tolerated by vorticellas and, therefore, were incorporated into the axenizing procedure.

Yeasts did not yield to the antibiotics and had to be removed by washing and dilution. Sterile vorticellas were finally obtained by transfer of groups of individuals through 6

washes. One of these, the 4 th, lasted 8 houre in the dilution of ampicillin-streptomycin.

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鏈形吊鐘蟲之無菌性純培養

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摘　要

本研究試驗之目的，在於尋求用適當之抗生素來殺滅與鏈形吊鐘蟲（*Vorticella monilata*）同時存在於培養基中之細菌等微生物（其中以具有動力且可形成孢子之革蘭氏陰性桿菌爲主），藉以經由此種處理而獲致該種原生動物之無菌性純培養。

本研究所用之吊鐘蟲係採自校園內之水池，經與其他原生動物分離而培養於用蛋黃、蒿苣調製成功之液態培養基（簡稱EL）中，在長達兩個月之繼續觀察與維護下，顯示此動物已在此時時更新之培養基中形成爲相當繁盛之一大族群，是爲帶菌性之純培養。爲瞭解此培養基中之細菌對何種抗生素最爲敏感，經作細菌培養及抗生素紙盤試驗（Disc test）之結果，得知Achromycin（Tetracycline HCL）及Polymyxin之效果甚佳，但進一步試驗之下，却發現這兩種抗生素對吊鐘蟲本身亦均有很大的毒性，故無實用價值。旋經試用Ampicillin與Streptomycin之混合劑，在適當濃度調配之下，乃可達到滅菌而不傷害吊鐘蟲之目的，經過連續六次之浸洗及稀釋處理後，（其中之第四次浸洗在含有Ampicillin與Streptomycin之稀釋液中曾持續達八小時之久，足以消除殘餘菌體中之孢子，而仍無損於吊鐘蟲之自由活動），終於獲得若干不帶菌而具有正常活力之吊鐘蟲，至於如何能長久維持此種動物於無菌性之純培養中，則有待未來進一步之研究。