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PHYTOPLANKTON CULTURE AS FEED - ISOLATION, IDENTIFICATION AND MAINTENANCE

C.P. GOPINATHAN

Tuticorin Research Centre of
Central Marine Fisheries Research Institute
Tuticorin-628 001.

INTRODUCTION

It is an established fact that the phytoplankton forms the primary or basic food of almost all the larval organisms of molluscs and fishes. In the natural environment, the larvae feeds on the phytoplankton available in the environment. In a hatchery, we have to isolate these organisms after identification and test them whether the feed is acceptable to the rearing larvae. Moreover, these organisms should have high protein content and they have to be maintained in good condition as stock cultures using various culture media and then supplied to the hatchery in required quantities.

CULTIVABLE SPECIES

Realising the importance of diatoms (Skeletonema costatum and Chaetoceros spp.) as the essential food of the prawn larvae and phytoflagellates as the food of the bivalve larvae, the process of isolation, identification, maintenance of stock culture and mass culture is imperative in the hatchery system throughout the world. The bivalve larvae

can ingest nothing larger than 10 microne and appear to rely for food on nannoplankton flagellates belonging to the algal Classes Haptophyceae and Chrysophyceae. Isochrysis galbana, a member of Haptophyceae has proved to be the ideal flagellate food for the rearing operations of both pearl and edible oyster larvae. Apart from the different strains of Isochrysis, species of Pavlova, Dicrateria and Chromulina were also found to be ideal food to the bivalve larvae during rearing operations.

ISOLATION

Isolation of the required organisms can be done by the following methods:

1. Pipette method: Large organisms can be pipetted out using a micro-pipette and transferred to culture tubes having suitable culture media.

2. Centrifuge or washing method: By repeated centrifuging of the sample and by inoculating the deposit, we may get different organisms.

3. By exploiting the phototactic movements: By this method, most of the phytoflagellates can be isolated. The water sample in a beaker is kept inside a dark chamber having a hole at one corner. A candle is placed near the hole. Since the flagellates have a tendency to move towards light, they will come to the surface near to the candle. By pipetting, we can separate these organisms and by tube culture method, they can be raised to a pure culture.

4. By using agar or agar plating method: For preparing the agar medium, 1.5 gm of agar is added to 1 litre of suitable medium or even natural seawater. This agar solution is sterilized in an auto-clave for 15 minutes under 15 lbs pressure and 120°C temperature. This

medium is poured in sterilized petri-dishes and keep for 24 hours.

The required species to be isolated, can be picked up by a platinum needle or platinum loop, under microscope and streaked on the surface of agar plate. After inoculation, these petri-dishes are placed in an incubation chamber for 7-8 days providing constant light (1 k lux) and temperature (25°C). Within this period if the required species, has grown into a colony, it is removed by a platinum loop and transferred to culture tubes. Further, from the culture tubes to small culture flasks and larger conical flasks, the algae can be cultured on a mass scale.

5. Dilution culture method: For the isolation of required species of phytoflagellates, the serial dilution culture technique is employed. In this method, mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} & 10^{-4}) or 4 steps 1, 10^{-1}, 10^{-2} & 10^{-3}) are required for the isolation of the flagellates (Sournia, 1971).

After filtering the seawater through 10 microne seive, the filtrate is inoculated to 4 series of culture tubes in various concentrations and kept under constant light (1 k lux) and temperature conditions (25°C). After 15 days, one can observe some discolouration of the culture tubes. On examination, the growth of a unialgal species could be noted in these tubes. Further purification of these organisms can be done by sub-culturing the same in 250 ml, 500 ml and 1 litre conical flasks. Finally if the culture is fully purified, it is transferred into 3 litre or 4 litre Haufkin culture flasks as stock culture.

CULTURE MEDIUM

For the successful culturing of micro-algae various chemical solutions have been recognised depending on the organisms, class and genera. Although all micro-algae are photo-autotrophic and can grow in purely inorganic media, many require organic compounds, the requirements of which may be either absolute or stimulatory. Usually, for culturing the micro-algae, Schreiber's medium or Miquel's medium were used in earlier days. However, for culturing the flagellates, Conway or Walne's medium is used for the maintenance of stock culture as well as for mass culture. Since this culture medium contains the chemicals, trace metals and vitamins (B_1, B_{12}) required for the micro-algae, the flagellates are being cultured by using this media alone (Gopinathan, 1982).

IDENTIFICATION

For maintaining the nannoplankters and flagellates as stock culture, first of all these organisms have to be identified and isolated. By noting the cell structure, shape of the cell, nature of cell walls, number of flagellae and movement of the organisms, the favourable ones can be identified. Most of the Haptophycean and Chrysophycean flagellates are having golden yellow colour, measuring less than 10 microns, round or oval shaped, with 1-3 flagellae and having wavy or rotatory movements. Silicified cell walls are the characteristic feature of diatoms while the flagellates are having cellulose cell walls. Further, a preliminary trial by feeding in a 10 litre beaker should be conducted to test whether the isolated organisms are acceptable to the rearing larvae.

STOCK CULTURE MAINTENANCE

Stock cultures of all the micro-algae are maintained in a special room having uniform temperature and light conditions. The cultures are kept in 3 or 4 litre Haufkin culture flasks. The autoclaved or heated seawater after cooling is poured to the culture flasks and required nutrients are added. Walne's medium enriched with vitamins is the ideal one suitable to maintain the stock of all the flagellates. About 10 ml of the inoculum in the growing phase of the culture is transferred to the culture flask and placed in front of two tube lights. For about 5-6 days, these flasks were kept exposed to two tube lights and when the maximum exponential phase has reached, only one tube light is needed for further growth. The time required to reach the maximum cell density will vary depending on the species. However, it was noticed that all the Haptophycean flagellates required 2 weeks for the completion of growth phase before entering into the stationary phase. In the stationary phase, the flagellates can be kept for a period of 2 months in the stock culture room under controlled condition of light and temperature, with or without aeration. A maximum of 5 Haufkin culture flasks required for keeping the stock culture of selected species. Periodical check-up is necessary to verify that the culture has not been contaminated. At least once in a month, all the species should be re-cultured to ensure viability and for better survival.

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