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Short Communication

Two Important Techniques for Isolation of Microalgae

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Abstract

Microalgae (phytoplankton) plays a very important role as live food for aquatic organisms. However, out of the 80,000 species of microalgae, only 50-60 species are commercially important as live food and for other nutritional purposes. Moreover, these microalgae are available in freshwater as well as marine water which should be isolated first and then used for monoculture or production for use as live food or for other commercial purposes. There are two important cosmopolitan methods worldwide for the isolation of microalgae and then pure culture. These two methods are: Micropipette Washing Technique and Centrifuge Washing and Streak Plating Technique which are described in detail for the isolation of microalgae in the Materials and Methods section, to make these methods available to researchers. So far, there is no available published literature about a reliable, easily understandable technique for the isolation of phytoplankton and or microalgae in our library nor in our country. Researchers as well as students are having a hard time obtaining the same from their localities. Therefore, with the aim in view of producing a manual discussing these two important techniques to isolate microalgae using clear and simple language and easily available, this paper is prepared.

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Introduction

It is widely recognized that microalgae can be used as feed for animals and may be a rich source of high quality protein supplement for human food (Burford and Preston 1994; Habib 1998). Their nutritional values have been substantiated by a large number of studies and compare well with conventional food products. The various biotechnological improvements that are now in progress will result to an increase in the productivity of microalgal cultures and a decrease in production costs (Sasson 1997).

The constant increase in aquaculture production and the intensification of the process have raised the need for a mass culture of particular commercial species and a larger supply of that particular microalgae than just the amount that can be harvested from natural habitats. At present, most hatcheries produce their own microalgae on site and some of them have developed the process of selling algal concentrate to other hatcheries. Therefore, there is no doubt that isolated important microalgae play an important role in aquaculture development. In this regard, the methods used are well known in different countries of the world but these are not well known and easily available to researchers in Bangladesh. It is very essential to publish appropriate methods for isolation of microalgae and make these available to researchers in the country. Hence, the present work was undertaken to discuss two important techniques (methods) for the isolation of microalgae.

Materials and Methods

Collection of microalgae

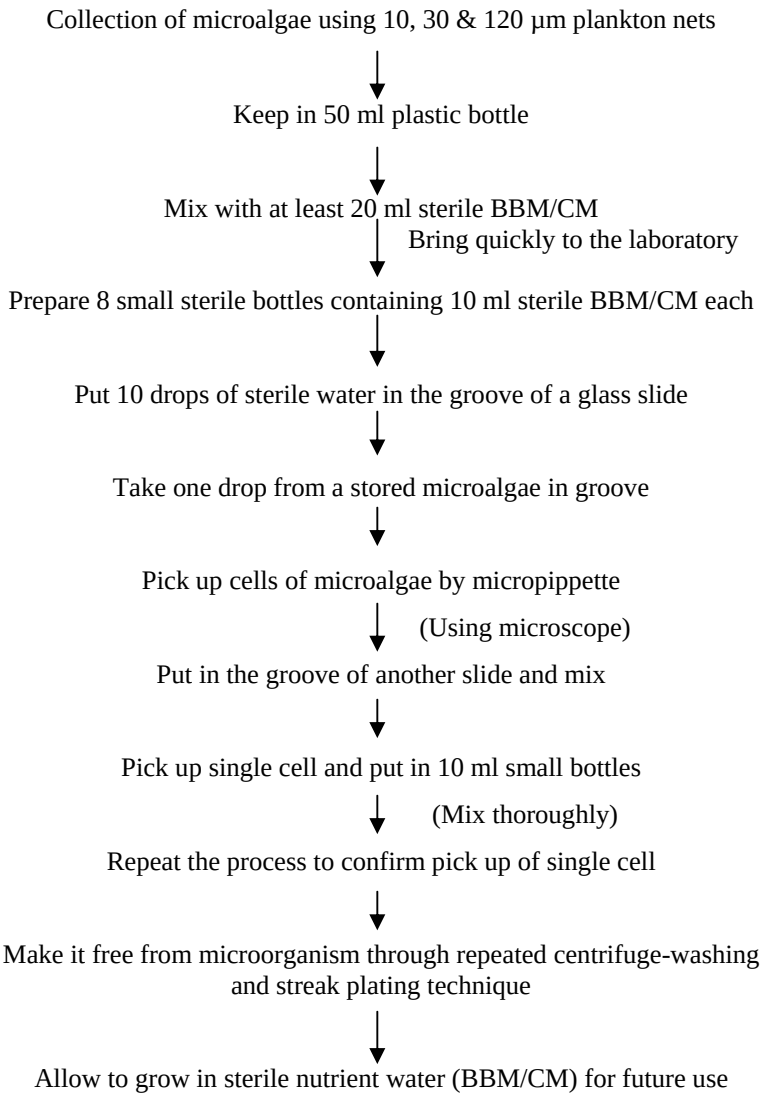
The microalgal (phytoplankton) samples will first be collected from ponds. Thirty liters of water will be collected from different depths of pond and pass through an upward series of plankton nets of mesh sizes 10, 30 and 120 µm (Bolting silk no. 55) to collect different useful species from the sample. The collected samples will be first taken in vials containing sterile nutrient solution (Bold basal medium for freshwater microalgae, and Conway medium for marine and coastal water microalgae). It is important to note that the collected microalgae should be kept in nutrient solution to keep them alive and to allow them to grow. Some samples will be concen-

trated to 20 ml, preserved in 10% buffered formalin (10 ml conc. formalin; 4.0 g NaH_2PO_4, H_2O; 6.50 g Na_2HPO_4 and volume made to 100 ml with distilled or deionized water) and transported to the laboratory for identification. The algae will be identified up to species to know the diversity and abundance, and counted using Sedgewick Rafter Counting Chamber (Boyd 1990) under an inverted divert microscope following the keys of Pennak (1978), Pontin (1978), Prescott (1984), Bold and Wynne (1978), Yamagishi (1992) and Vymazal (1995). Among the established techniques used for the isolation of microalgae, the following two important cosmopolitan methods are described.

Micropipette Washing Technique (Phang and Chu 1999)

Preparation of micropipette: The tip of a glass pasteur pipette will be heated first in a flame and stretched when it has become soft. The tip of the pipette will be broken using a pair of fine forceps. The micropipettes will be plugged with some cotton, sterilized at 126°C for 15 minutes and attached to a piece of soft rubber tubing when ready for use. These will be used to pick up single cells from the collected sample under a microscope.

Washing Technique: The collected microalgal samples will be put in either sterile Bold basal medium (BBM) (for freshwater algae) or Conway medium (CM; for marine algal sample) right after collection. A micropipette will be used to pick up individual cells through repeated try and error method. Ten drops of sterile BBM or CM will be placed in the groove of a glass slide. Then a drop of the microalgal sample will be added and observed under an inverted microscope. Having confirmed the target organism, one drop of the sample will be transferred to the next one. The procedure will be repeated several times so as to wash it with sterile water and to isolate single cell in pure form and to make it free from contamination (Hoshaw and Rosowski 1973; James 1978; Guillard 1995). Thus the isolated microalgal species will be allowed to grow in either BBM or CM and maintained in the laboratory. For their propagation and long time preservation, the single cell will also be put in agar plate, broth culture and test tube in the laboratory. This technique may not be highly appropriate because it is not possible to make the single cell free from contamination, so streak-plating technique should be followed to get a bacteria free single cell. For easier understanding, the procedure is expressed through this flow chart:



Centrifuge Washing and Streak Plating Technique (Phang and Chu 1999)

Preparation of agar plate: Agar plates will be prepared by dissolving 2% agar (w/v) in either BBM or CM. These plates will be autoclaved at 126ºC for 15 minutes. Then these plates will be allowed to cool down and 5 ml warm agar medium will be put in the plates. These plates will be

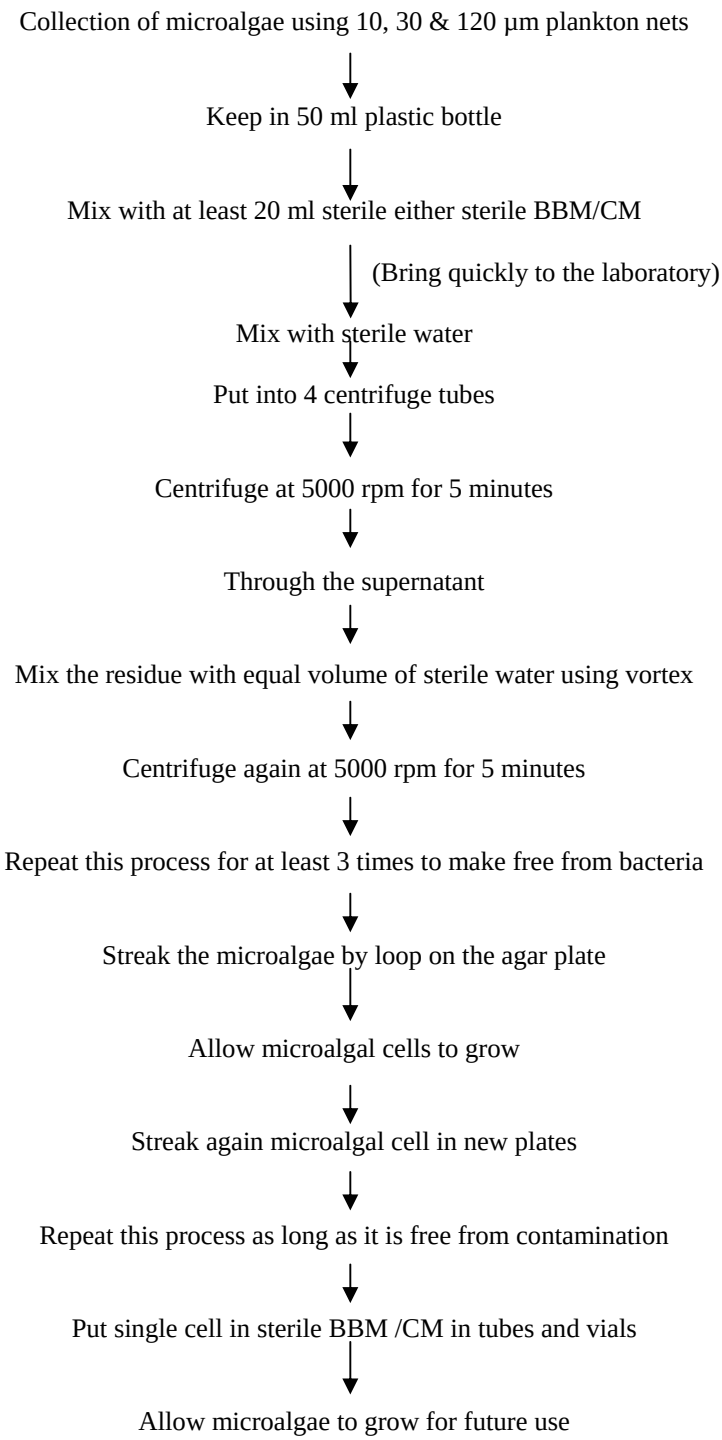
allowed to cool, kept in inverted position for not drying and at least 72 hrs before streaking.

Centrifuge Washing Technique (Purification of algal samples)

A volume of 12 ml microalgal sample will be taken especially from enrichment culture in each of at least four centrifuge tubes. These tubes will be centrifuged at 3000 rpm for 15 minutes. After removing the super-natant, the cells will be suspended in fresh sterile water in each tube using vortex mixer (rotated at 1000-1500 rpm up to homogeneous suspension). About 500 ml sterile water will be prepared using autoclave (at 126ºC for 15 minutes) to complete centrifuge-washing process. Centrifugation and washing will be repeated for six times to expel most of the microorganisms presented in algal sample and the cells will be then streaked on to agar plates.

Streak Plating Technique (Phang and Chu 1999)

Washed microalgae will be allowed to streak through loop in plates in axenic condition and to keep for at least seven days to grow microalgae. Repeated streak-platings will be carried out to peak up single colony from earlier streaked plates and to make free from bacteria. From last streaked plates, the single colonies will be picked up by loop and allowed to grow in tubes and vials. Before putting in the tubes and vials, the single cell growth and purity of single species will be confirmed after observing under micro-scope. Then, the pure culture of isolated microalgae will be maintained in BBM in tube, vial and volumetric flask in the laboratory for further use and study. Following the above procedure, the important microalgae will be isolated, allowed to grow in BBM and maintained in the laboratory. For easy understanding the process is expressed through the following chart:


Collection of microalgae using 10, 30 & 120 µm plankton nets
Keep in 50 ml plastic bottle
Mix with at least 20 ml sterile either sterile BBM/CM
(Bring quickly to the laboratory)
Mix with sterile water
Put into 4 centrifuge tubes
Centrifuge at 5000 rpm for 5 minutes
Through the supernatant
Mix the residue with equal volume of sterile water using vortex
Centrifuge again at 5000 rpm for 5 minutes
Repeat this process for at least 3 times to make free from bacteria
Streak the microalgae by loop on the agar plate
Allow microalgal cells to grow
Streak again microalgal cell in new plates
Repeat this process as long as it is free from contamination
Put single cell in sterile BBM /CM in tubes and vials
Allow microalgae to grow for future use

Discussion

These two techniques are not so easy to follow and to complete the work smoothly. The work should be done very carefully. However, the pure culture will not be free from contamination when Micropipette Washing Technique will be used. To make the isolated microalgae free from contamination, Centrifuge Washing Technique should be used for freshwater, and marine as well as coastal water samples (Phang and Chu 1999). It is hopeful that these two techniques will be easily available to researchers and scientists for their work.

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