Lab.2: Protocols for Isolation and culturing of algae

In phycological research, there is nothing more basic and essential than culturing algae. First, we have to know that there are three main types of algal culture as mentioned bellow:

1-**Mixed algal culture**: this type of culture is containing more than one algal species

2-**uni-algal culture**: this type of culture is containing only one algal species usually a clonal population (but which may contain bacteria, fungi, or protozoa)

3-**Axenic culture**: this type of culture is containing only one algal species without contaminated bacteria, fungi, or protozoa.

Two methods to **isolate algae** or **purify contaminated algal cultures** and /or **to produce single cells** are described below: agar plate method, and picking up of single cells from the original culture by using the capillary method.

A-Isolation of algal strain by agar:

1-prepare Serial dilutions from the collected samples in sterilized tubes starting with 1 ml of sample added to 9 ml of sterilized distilled water. The series of dilution was ranged from 10^{-1} to $10^{-10.}$

2-take a loop full from different dilutions to prepare a slide, and examined under light microscope**(40x)** to determine the type of algae which observed in the certain sample.

3- Prepare several "isolation plates". These are simply agar plates prepared with the growth medium you are using to grow the algae

4-After the target dilution was examined by microscope, transfer 0.1 ml from each of the dilution tube and spread with a sterile glass spreader(or streaking by sterile loop) across the surface of agar plates of medium prepared previously. Triplicate plates were prepared for each dilution to isolated cyanobacteria, and incubated **in**

illuminated incubator at **(26±1°C and illumination intensity about 200 Micro Einstein (µE) / m² / Sec for a period of 16 to 8 hours Lighting: darkness. with shaking).**

Advantages of shaking(agitation):

1-to obtain better mixing of nutrients as well as better distribution of light

2-to obtain good aeration resulting in better gas solubility, thus, increase in biomass yield.

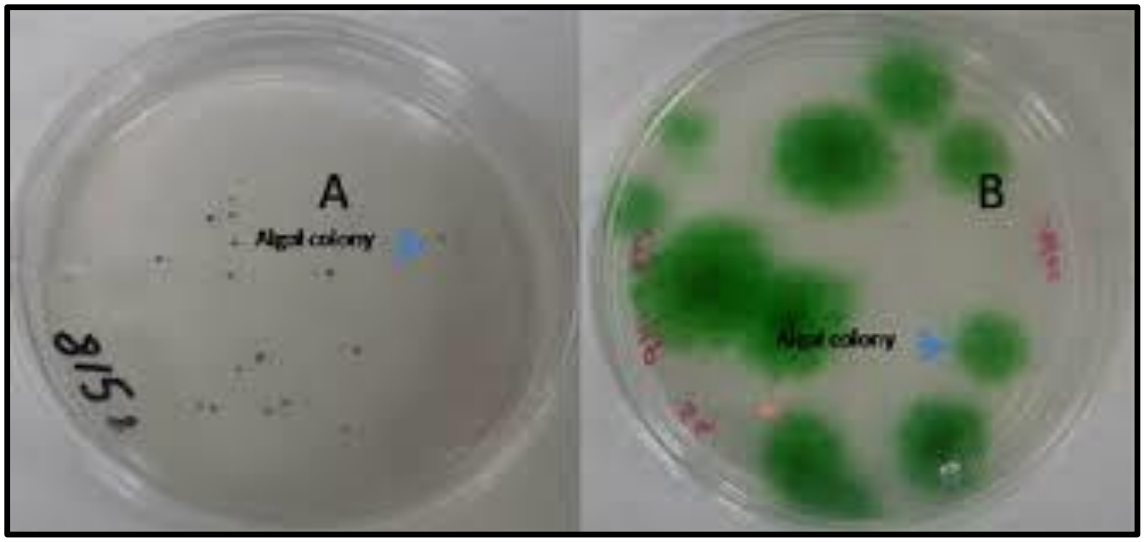

Figure (1): isolation of algae by agar plating method

B-Picking up method (Capillary method): This technique is follows the dilution method, but the inoculum is obtained by selecting **single cells, colony, or filament** of the desired species by means of a **capillary pipette** handled under a microscope, then transferring to a new test tube containing sterilized suitable media.

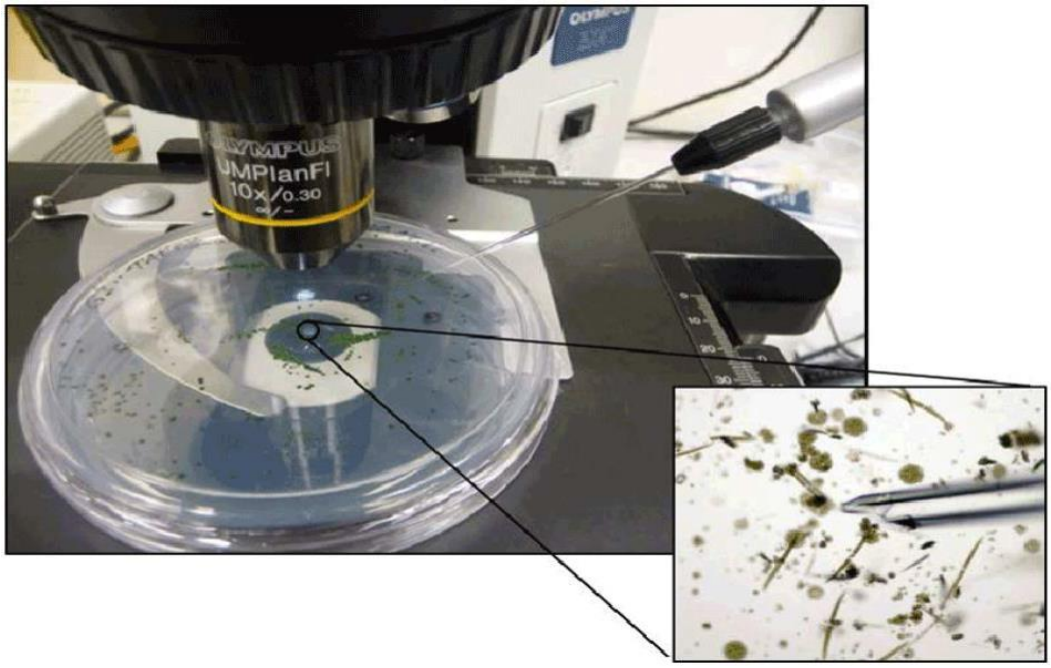

Figure (2): isolation of algae by capillary method

Algal media

Algal media refers to the solution nor culture in which algae grow. All the media have several components in common: sources of nitrogen (in the form of nitrate, nitrite and ammonia), phosphorus, vitamins and trace metals. However, the specific types of these nutrients, their concentrations and ratios vary between the media. There are many types of algal culturing media, but we will list the most common media as mentioned bellow:

1- **F/2 Medium (for marine algae)**

2- **Chu's Medium No. 10** and **BG-11medium (for fresh water algae)**

Note: Chu-10 is used for the enhancement of most algal species growth. While BG-11medium used for the enhancement of algal growth especially blue-green algae.