Lab.1: Protocols for Isolation and Purification 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.

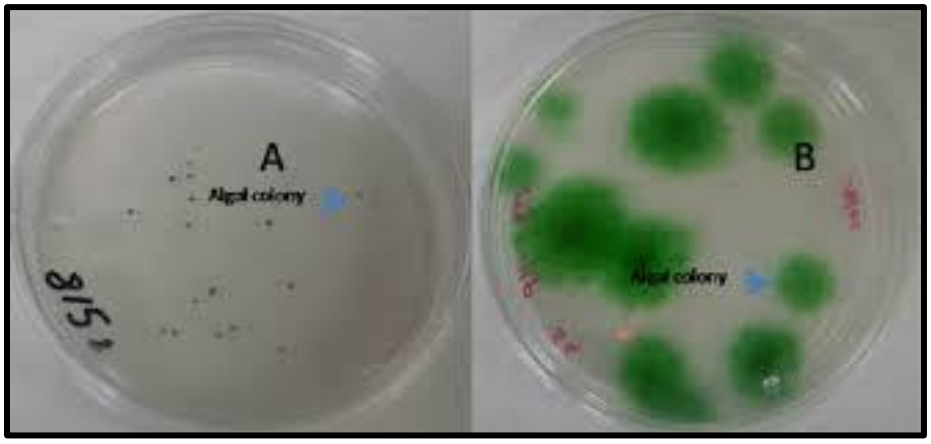
Two methods to **isolate algae** or **purify contaminated algal cultures** and /or **to produce single cells** are described below: agar plate method, and picking up of single cells from the original culture by using the capillary method.

A-Isolation of algal strain by agar:

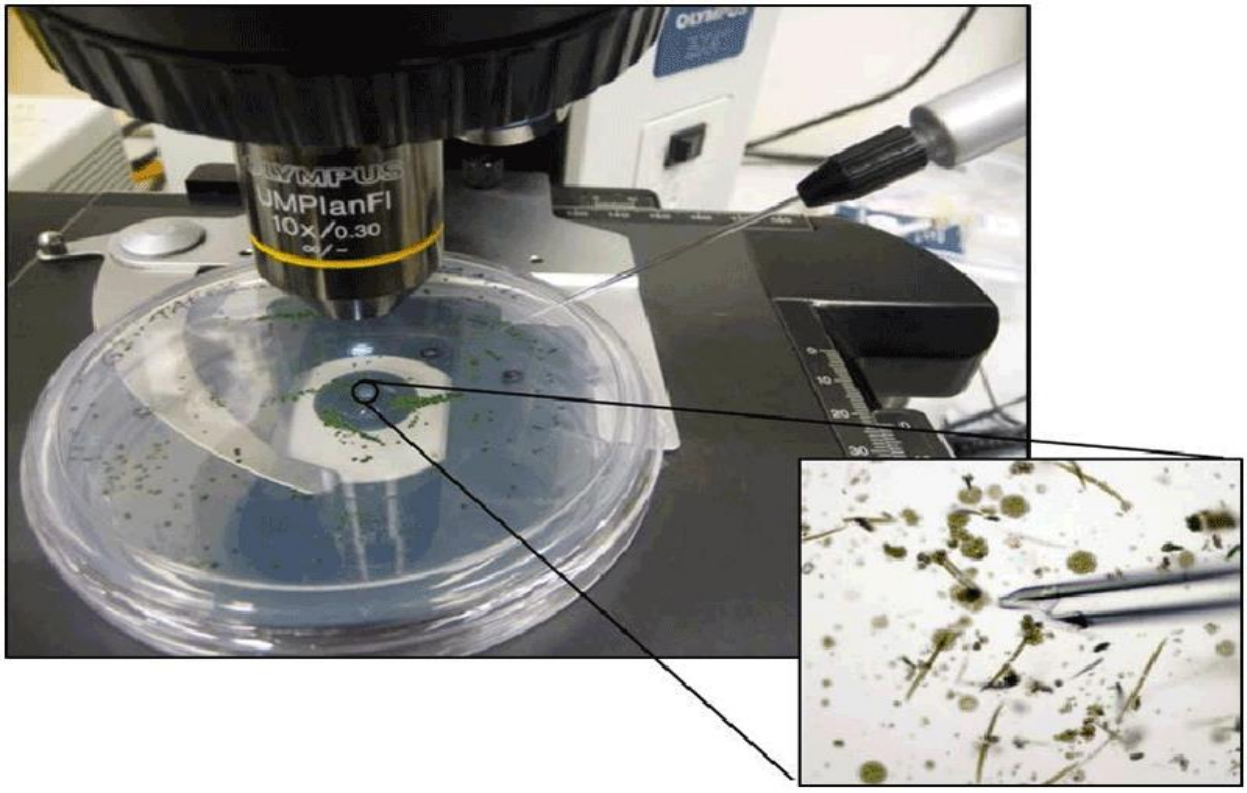
1-prepare Serial dilutions from the collected samples in sterilized tubes starting with 1 ml of sample added to 9 ml of sterilized distilled water. The series of dilution was ranged from 10^{-1} to $10^{-10.}$

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 algae4-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).**



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.



After the appearance of aggregated colonies on the plates surfaces. or color change in cultivated test tubes, examine algal isolates under light microscope (40X) to determine the desired isolates**, uni -algal culture** was obtained by using the following methods:

A-Several successive transfers.

The isolated algae, sequentially sub cultured several times on other plates and incubated. Each subculture was examined frequently; this method was repeated till a unialgal culture have been obtained.

B-Single filament isolation.

This technique depends on gliding motility and photo taxis of filamentous algal species. The cultures which are grown on agar plates transferred to new one to examine the ability of filaments to grow and move toward a single source of light to pick a single filament of algal species. Once the single filament moved a sufficient distance on agar, it was removed under sterilized conditions and transferred into a flask contained fresh liquid medium.