1 Isolation techniques

Follow the sample collection protocols below for any environmental sample from which you hope to isolate photosynthetic microbes. Whenever possible, the plate-spreading method is the preferred strategy to isolate clean monocultures.

1.1 Plate-spreading isolation method

1. Agar medium is prepared with 1-1.5 % agar in 1 L of the suitable algal cultivation medium (TAP, BG-11, F/2 and/or Zarrouk), media is autoclaved to melt agar and make sterile, if an alga is sensitive, agar can be autoclaved separately and mixed with sterilized liquid culture medium. Molten agar-media are poured into sterilized Petri dishes.
2. For samples which already indicate the presence of microalgae (by green or other coloration), dilute 30-50 µL of the sample into 700 µl of respective medium and spread onto agar plate. The greener the medium, the less you will spread;
3. Spread the sample evenly over the surface of agar using the L-shaped spreader and let dry;
4. Incubate under low moderate lights (24h of light) at ~25-30°C for at least 4-7 d and if possible, 1-3% CO_2 in air supplementation. Spread plates will have a countable number of colonies evenly distributed, some with different phenotype if mixed starting culture was present.
5. It is important to pick only single colonies from the plates and then place these in individual wells of a 24- well culture plate with 1.5 mL of respective culture medium.
   *You can also use a 96- well plate with 0.2 mL of culture medium.;
6. Incubate under low-moderate lights (24h of light) at ~25°C for at least 7 days, with agitation and if possible, 1-3% CO_2 in air supplementation.
7. After this, drop a sample of each well on a glass slide to check microscopically if you have algal monoculture.
8. If you have monocultures, you can "scale up" the sample from each well to 6 well plates and then transfer it into larger flasks or filter-cap Tissue Culture (TC) bottles to maintain your monoculture in liquid.
9. A further backup is made by concentrating the culture (either by centrifugation or pipetting) and spreading it on an agar slant which is maintained in low-light.

10. Liquid cultures in TC flasks and agar slants can be let in low-light conditions usually for a month before refreshing.

1.2 Serial dilution

1. Pre label 10X 15 mL tubes 10^{-1}–10^{-10} to indicate dilution factor, fill 9 mL of target algal liquid medium into each;
2. For samples that visibly contain algae (green coloring), aseptically pipette 1 mL of enrichment sample to the test tube (10^{-1}) and mix gently to make a 1/10 dilution. This procedure is repeated for the remaining tubes to enact the serial dilution (10^{-2}–10^{-10}), *15 mL centrifuge tubes work, however glass can prevent adhesion to vessel walls.
3. Incubate all tubes under low moderate lights (24h of light) at ~25°C for at least 7 d, under gentle agitation and if possible, 1-3% CO_2 in air supplementation.
4. Examine cultures within tubes microscopically after the incubation period. A monoculture culture may grow in one of the tubes with higher-dilution (10^{-6}–10^{-10});
   *If the tubes contain two or three different species, then micromanipulation or dilution plating can be used to obtain monocultures.
5. If you have monocultures, you can "scale up" the sample from each well to 6 well plates and then transfer it into larger flasks or filter-cap Tissue Culture (TC) bottles to maintain your monoculture in liquid.
6. A further backup is made by concentrating the culture (either by centrifugation or pipetting) and spreading it on an agar slant which is maintained in low-light.
7. Liquid cultures in TC flasks and agar slants can be let in low-light conditions usually for a month before refreshing.

1.3 Micromanipulation

1. A micromanipulation pipette is made by heating on flame and pulling a glass capillary tube out from both ends. The point of the tip should be at least twice the diameter of the cell to be manipulated;
2. For samples that visibly contain algae (green coloring), while viewing the sample under a microscope, suck individual cell(s) into the micropipette. Transfer the cell to a drop of sterile

medium on a glass slide, wash or sterilize the micropipette in flame or with ethanol and rinse with medium, picked the cell from the drop and repeat in a new drop. This process is aimed to “wash” the cell from bacteria;

*The more times a cell is washed, the less likely is bacterial contamination. However, the risk of cell damage increases with the number of times a cell is handled.

3. Then transfer the cell to a 24-well plate or the surface of an agar plate of appropriate medium;
4. Incubate under low moderate lights (24h of light) at ~25°C for at least 7 days with low lights preferred until the cell can establish itself and replicate in its new medium. Gentle agitation can be used for the liquid samples and if possible, 1-3% CO_2 in air supplementation for both agar plates and liquid cultures.
5. If you have monocultures, you can "scale up" the sample from each well to 6 well plates and then transfer it into larger flasks or filter-cap Tissue Culture (TC) bottles to maintain your monoculture in liquid.
6. A further backup is made by concentrating the culture (either by centrifugation or pipetting) and spreading it on an agar slant which is maintained in low-light.
7. Liquid cultures in TC flasks and Agar slants can be let in low-light conditions usually for a month before refreshing.

2 Media recipes

General Solutions

TAP –Medium

Components	1 L	2 L	3 L	4 L	5 L
TRIS	2,42 g	4,84 g	7,26 g	9,68 g	12,1 g
TAP-Salts	25 ml	50 ml	75 ml	100 ml	125 ml
p-Solution	0.375 ml	0.75 ml	1.125 ml	1.5 ml	1.875 ml
Hutner's trace	Each solution:1 ml	Each solution:2ml	Each solution:3 ml	Each solution:4 ml	Each solution:5 ml
Acetic Acid (100%)	1 ml	2ml	3 ml	4 ml	5 ml

pH to 7,0 with HCl

TAP-Medium (–Sulfur, –S)

All components are the same as regular TAP, but
ATTENTION (!!!) for sulfur free medium (-S) The following solution must be substituted:
TAP salts (–S)* and for the **Hutner's trace solutions, use $ZnCl_2$** (instead of $ZnSO_4$).

TAP Salts solution (1L)

16 g NH_4Cl
4g $MgSO_4 \times 7\ H_2O$
2g $CaCl_2 \times 2\ H_2O$

***TAP- Salts (–S)** (1 L)

16 g NH_4Cl
3.3 g $MgCl_2 \times 6\ H_2O$
2g C $aCl_2 \times 2\ H_2O$

TAP(-N) Salts solution (1L)

20.9 g KCl
4g $MgSO_4 \times 7\ H_2O$
2g $CaCl_2 \times 2\ H_2O$

TAP (NO_3 - Nitrate) Salts solution (1L)

23.8 g $NaNO_3$
4g $MgSO_4 \times 7\ H_2O$
2g $CaCl_2 \times 2\ H_2O$

Phosphate-solution for TAP- Medium (aka **P-solution**) (100 ml)

14,8 g KH_2PO_4
28,8 g K_2HPO_4

Hutner´s trace- Pre-Solutions

Stock solution 1: 125mM EDTA- Na_2
13,959 g EDTA- Na_2 in 300 ml
(pH to 8,0 with KOH)

Stock solution 2: 285µM $(NH_4)_6MO_7O_{24}$
0,088 g $(NH_4)_6MO_7O_{24}$ in 250 ml

Stock solution 3: 1mM Na_2SeO_3
0,043 g Na_2SeO_3 in 250 ml

Hutner´s trace- Solutions

1. **EDTA – Na_2**
20 ml **Stock solution 1**: fill to 100 ml with ddH_2O

2. **$(NH_4)_6MO_7O_{24}$**
10 ml **Stock solution 2**: fill to 100 ml with ddH_2O

3. **$Na2SeO_3$**
10 ml **Stock solution 3:** fill to 100 ml with ddH_2O

4. **Zn · EDTA**
0,072 g $ZnSO_4 \cdot 7H_2O$ + 2,2 ml **Stock solution 1**: fill to 100 ml with ddH_2O
For TAP- S
0,017 g **$ZnCl_2$** + 1,1 ml **Stock solution 1**: fill to 100 ml with ddH_2O

5. **Mn · EDTA**
0,1188 g $MnCl_2 \cdot 4H_2O$ + 4,8 ml **Stock solution 1**: fill to 100 ml with ddH_2O

6. **Fe · EDTA**
0,82 g EDTA-Na_2 (**Not from Stock Solution 1!!**) + 0,232 g Na_2CO_3 mix into water and add:
+ 0,54 g $FeCl_3 \cdot 6H_2O$ and fill to 100 ml with ddH_2O

7. **Cu · EDTA**
0,034 g $CuCl_2$ + 1,6 ml **Stock solution 1**: fill to 100 ml with ddH_2O

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HSM- Medium (1 L)
50 ml Beijernick's Solution
+ 1 ml from each of the 7 Huntner's trace solutions
Fill to 975 mL with ddH_2O
ph to 6.8
Autoclave
After cooling, add 25 ml HSM P-solution (below) sterile.

Phosphate-solution for HS-Medium (HSM) (1 L) (HSM P-solution)
29,6 g KH_2PO_4
57,6 g K_2HPO_4

Beijernick's solution (1 L)
10 g NH_4Cl
0,4 g $MgSO_4 \times 7\ H_2O$
0,26 g $CaCl_2 \times 2\ H_2O$

BG-11

1. Stock Solutions

Solutions	Chemical Composition	Amount ($g.L^{-1}$)
Stock 1	Ferric ammonium citrate	0.6
	Citric Acid	0.6
	$CaCl_2 . 2 H_2O$	3.6
Stock 2	$MgSO_4 . 7 H_2O$	7.5
Stock 3	$K_2HPO_4 . 3 H_2O$ or K_2HPO_4	4.0 or 3.05
Stock 4	$NaNO_3$	100
Stock 5	H_3BO_3	2.86
	$MnCl_2 . 4 H_2O$	1.81
	$ZnSO_4 . 7H_2O$	0.222
	$CuSO_4 . 5H_2O$	0.079
	$COCl_2 . 6H_2O$	0.050
	$NaMoO_4 . 2H_2O$ or MoO_4 (85%)	0.391 or 0.018

All stock solutions must be sterile filtered or autoclaved.

2. For basic BG11 medium combine the following stock solutions

Solutions	Per liter to be prepare
Stock 1	10 mL
Stock 2	10 mL
Stock 3	10 mL
Stock 4	10 mL
Stock 5	1 mL
Na_2CO_3	0.02 g

Combine all stock solutions and fill until 1 L with fresh water and adjust pH to 7.5 (use 1M HCl);
After autoclaving and cooling the pH is about 7.1.;
For solid media add 1.5%agar.

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Guillard's (F/2) medium

Guillard's (F/2) Marine Water Enrichment Solution - Sigma Aldrich
Seawater (Red Sea Water)

3 References

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