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We use this protocol and it's working

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Cyanobacteria growth (English)

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ABSTRACT

This document aims to present a comprehensive protocol for the cultivation of cyanobacteria in saline aquatic environments. The protocol incorporates etailed information on the composition of solutions, fundamental calculations, and required volumes for the successful execution of the entire procedure. It is pertinent to note that growth rates may exhibit considerable interspecific variations among different strains of cyanobacteria.

SAFETY WARNINGS

Autoclave:
Personal Protection: Always wear appropriate protective clothing, such as apron, gloves, safety goggles, and closed shoes when operating the autoclave.
Work Conditions: Ensure that the work area is well-ventilated and has easy access to emergency showers and eye wash stations.
Cooling: After autoclaving, wait for the cooling cycle to complete before opening the autoclave. High pressure and temperature can cause explosions if the process is not properly completed.
Caution with Steam: Be aware of the hot steam released when the autoclave is opened. Keep your face and hands away from the opening.
Maintenance: Ensure that the autoclave is in good working condition and has undergone regular maintenance.

Chemicals:
Safe Handling: Use appropriate gloves and exercise caution when handling chemicals. Avoid direct contact with skin and eyes.
Filtration: When using filters, follow guidelines for the safe disposal of contaminated filters.

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Storage: Store all chemicals according to safety regulations and in designated chemical storage areas.

Cotton Plugs:
Careful Handling: Handle cotton plugs carefully and ensure there are no chemical residues or contaminants on them.
Careful Autoclaving: When autoclaving cotton plugs, follow autoclave instructions carefully to avoid overheating or explosions.
Proper Drying: Ensure that the cotton plugs are completely dry before using them in Erlenmeyer flasks.

General:
Proper Procedures: Strictly follow all the steps and procedures described in the protocol. Do not take shortcuts and avoid improvisation.
Training: Individuals performing these procedures should be trained in laboratory safety and familiar with the associated risks.
Emergency Plan: Be aware of the laboratory's emergency plan and know how to act in case of accidents.
Safe Disposal: Ensure to dispose of chemical wastes and biological materials according to local regulations and laboratory guidelines.
Documentation: Keep accurate records of all steps performed, including dates and relevant details.

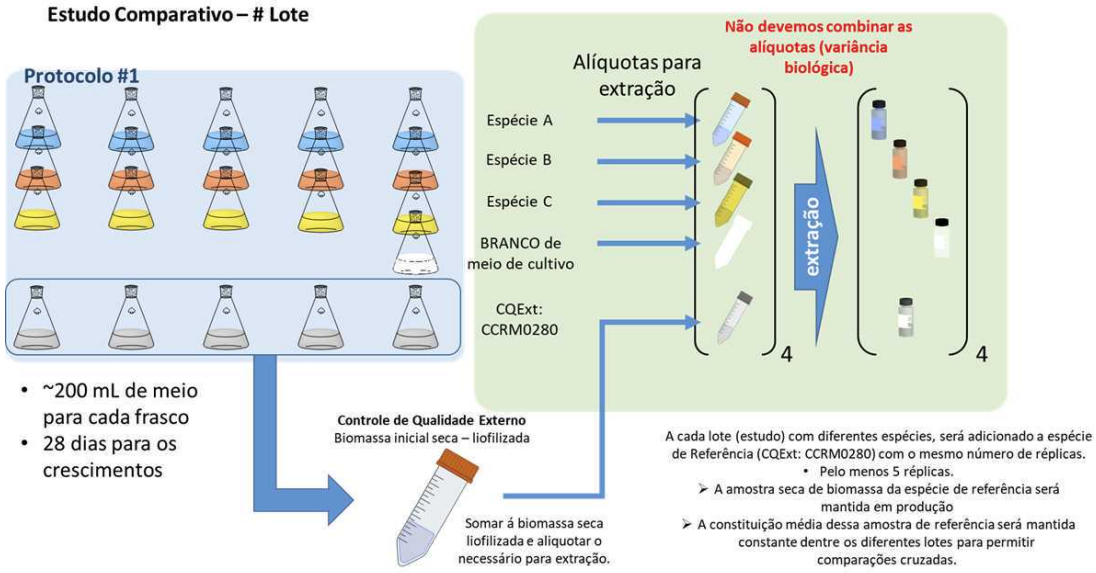
Remember that safety precautions may vary according to local regulations and the level of risk associated with the work performed in the laboratory. Therefore, it is essential to consult the specific safety guidelines of your workplace and follow best safety practices at all times.

Preparation Prior to Cultivation

1 The quantity of 500 mL Erlenmeyer flasks needed will vary based on the number of samples to be produced. Follow this plan:

- For X samples, prepare them in quintuplicates (multiply by 5).
- Prepare the External Quality Control Strain (CCRM0280) also in quintuplicates.
- Include 2 blank culture medium samples in Erlenmeyer flasks, treated identically with medium and nutrients, but without the inoculation step.

As an example, if comparing 3 different species, you would need a total of (3×5)+5+2=22(3×5)+5+2=22 flasks.



2 Filling Out the Metadata File:

- Sample codes
- Strain codes
- Species
- Culture media
- Start date
- Dates of nutrient addition
- Biomass collection date
- Extraction method (medium polarity: Dichloromethane-Methanol)
- Extraction method 2 (high-polarity: Methanol-Water)
- Comments

Remember to follow these steps for a complete and accurate record of the information related to the experiment.

Material

3

- 500 mL Erlenmeyer flasks
- 250 mL and 1 liter Scotch bottles
- 50 mL Falcon-type centrifuge tubes
- Sterile loops
- Reagents for preparing stock solutions (see below)
- Refrigerated Benchtop Centrifuge (Hettich Model 320R, Tuttlingen, Germany)
- SpeedVac (Christ model RVC 2-25, Osterode am Harz, Germany) at LabMeta - Chemistry Institute - UFRJ
- Lyophilizer (model L120, Liotop, Brazil)

4 Preparation of Stock Solutions:
Stock Solution of NaNO3:
Use a properly washed flask (previously washed with 5% extran and rinsed with distilled water until exhaustion)

- Add 7.5 g of NaNO3 (NEON, p# 01813) to 100 mL of distilled water
- Store the solution under refrigeration in a refrigerator

Use: 1 mL for each liter of medium.

5 Stock Solution of NaH2PO4•H2O:
Use a previously washed flask (previously washed with 5% extran and rinsed with distilled water until exhaustion)

- Add 0.5 g of NaH2PO4•H2O (NEON, p# 01415) to 100 mL of distilled water
- Store the solution under refrigeration in a refrigerator

Use: 1 mL for each liter of medium.

6 Trace Metal Stock Solution:
Use a washed flask (previously washed with 5% Extran and rinsed with distilled water until exhaustion).

- Add the following components:
- 23 mg of ZnSO4•7H2O
- 152 mg of MnSO4•H2O
- 7.3 mg of Na2MoO4•2H2O
- 14 mg of CoSO4•7H2O
- 6.8 mg of CuCl2•2H2O
- 4.6 g of Fe(NH4)2(SO4)2•6H2O
- 4.4 g of Na2EDTA•2H2O

- Top up the volume with distilled water to 1 liter.
- Store the solution under refrigeration in a refrigerator.

Use: 1 mL for each liter of medium.

7 Vitamin Stock Solution:

Use a flask that has been previously washed (previously washed with 5% extran and rinsed with distilled water until exhaustion).

- Add the following components:
- 200 mg of Thiamine
- 10 mL (0.1 g) of Biotin
- 1 mL (1 g/L) of Cyanocobalamin
- Top up the volume with distilled water to 1 liter.
- Store the solution under refrigeration in a refrigerator.

Use: After autoclaving the medium, add 1 mL for each liter of medium.

8 Preparation of Nutrient Stock Solution:

- Mix:
- 20 mL of NaNO3 Stock Solution
- 20 mL of NaH2PO4.H2O Stock Solution
- 1 mL of Vitamin Stock Solution
- 1 mL of Trace Metal Stock Solution
- Filter using a 0.22 µm filter

- Store the solution under refrigeration

9 Preparation of Cotton Plugs:

Materials needed: hydrophobic cotton, gauze, scissors, string.

- Cut a piece of gauze the size of the palm of your hand.
- Open the gauze and insert part of it into the flask.
- Place the hydrophobic cotton inside the gauze that is in the mouth of the flask.
- Continue inserting cotton until, when removing the gauze with the cotton from the flask, a "puff" sound is heard (indicating the vacuum caused by the Plug).
- Cut a piece of string.
- Tie the gauze with the string, ensuring that it is well above the cotton (without leaving space).
- Cut off the excess string and gauze.
- Check if the Plug is slightly above the mouth of the glass.
- If necessary, adjust its position.
- Autoclave: 15 minutes at 121°C.
- Dry the flask capped with the Cotton Plugs in an oven for 8-12 hours at 70°C.

Ensure that each step is followed precisely to ensure the quality of the culture medium and subsequent procedures.

Cotton Plugs are reused from one cultivation to the next. At the end of cultivation, it is sufficient to dry them in an oven to prevent the proliferation of fungi.

10 Preparation of F/2 Culture Medium:

- For 1 liter of distilled water, add:
- 41.5 grams of marine salt for aquarium (Reef Salt)

- 1 mL of NaNO3 Stock Solution
- 1 mL of NaH2PO4•H2O Stock Solution
- 1 mL of Trace Metal Stock Solution
- Autoclave: 15 minutes at 121°C
- Wait for the medium to cool down.

- Add 0.5 mL of the Vitamin Stock Solution before use.

Preparation Prior to Cultivation

11 Separate all the 500 mL Erlenmeyer flasks
- Wash them with 5% Extran.
- Rinse them thoroughly with distilled water.

It's important to consider the number of strains and replicates to be cultivated.

12 Prepare the Cotton Plugs to seal the cultures.

13 Autoclave the previously washed flasks with the Cotton Plugs
- Autoclave: 15 minutes at 121°C.
- Dry the flask capped with the Cotton Plugs in an oven for 8-12 hours at 70°C.

14 Autoclave the flasks containing the F/2 culture medium
- For 22 flasks: 22 x 200 ml = 4.4 liters (rounded up to 5 liters).
- Prepare the solution in a 5-liter flask and divide into 1-liter Schott bottles.

- Autoclave: 15 minutes at 121°C.

15 After the culture medium has cooled, add 0.5 mL of vitamin solution (previously filtered through a syringe filter: 0.22 µm)
- Note: Do not use the 0.45 µm syringe filter.

16
- Label each Erlenmeyer flask with tags containing a code (traceable in the project's sample list) and the production date.
- The production dates for each specimen, addition of nutrient solution, and biomass collections should be documented in the project's sample list.
- Ensure that each step is performed meticulously to ensure a proper start to the cultivation and reliable tracking of subsequent processes.

Inoculation

17 Using sterile loops, proceed to collect biomass from a previously prepared cultivation

- Quantity: a "tuft" approximately the size of a 50-cent coin.
- It is imperative to carry out all operations using disposable gloves and to minimize exposure to airflow.
- Properly seal the Erlenmeyer flasks with the Cotton Plugs prepared earlier.

Cultivation Maintenance

18 To maintain the cyanobacteria in the logarithmic phase, it is essential to provide frequent feeding through the addition of the previously prepared Nutrient Stock Solution.

- For each cultivation, it is recommended to add 200 µL of the Nutrient Stock Solution.

The need for nutrients in cyanobacteria can be visualized by their characteristic green color.

Biomass Collection and Treatment

19 The cultivation period is set at:

- 28 days.
- With the addition of the Nutrient Stock Solution at every 7-day interval.

20 At the end of the 28 days, from each Erlenmeyer flask, an amount corresponding visually to 80-90% of the biomass will be collected.

- We will allow each specimen to continue its growth until it becomes necessary to interrupt it.
- Initially, we can set a limit of 4 months for each specimen.

21 Transfer the culture medium from each Erlenmeyer flask with the biomass to a properly labeled 50 mL conical centrifuge tube (Falcon type), with the label corresponding to the reference code in the sample list.

- Centrifuge: 3011 xg (4500 rpm) for 15 minutes at 4°C
- Discard the supernatant
- Continue transferring the contents of each Erlenmeyer flask, centrifuging and discarding the supernatant until complete elimination of all the aqueous content
- Record the volume of biomass obtained (photo for file)

22 Subject each properly identified sample to a lyophilization process until dry.

- Each sample should be separately identified in clean, semi-screwed Falcon tubes (to prevent cross-contamination of the samples).
- Insert pertinent details and observations in the Metadata.
- Always store in a freezer at -20°C.