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Co-cultivation Protocol for *Porosira glacialis* Diatom and Bacterial Isolates

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We use this protocol and it's working

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Disclaimer

This protocol was developed as part of a master's thesis affiliated with **Microalgae & Microbiomes - The M2 Research Group** at **Norges fiskerihøgskole**, University of Tromsø, Norway

Abstract

The protocol provides a platform for controlled experimentation on axenic and paired cultivation of the cold-adapted marine diatom *Porosira glacialis* UiT201 diatom with its bacterial associates in a synthetic consortia under variable environmental conditions, allowing the characterization of diatom-bacteria growth dynamics. Treatments include UIT201-*Sulfitobacter marinus* co-culture and UIT201 treated with Indole-3-Acetic Acid (IAA); however, this protocol can be adapted to various algal strains, bacterial isolates, and conditions to study diatom-bacteria growth dynamics. Expected results include growth curves from measured cell density and chlorophyll a extraction.

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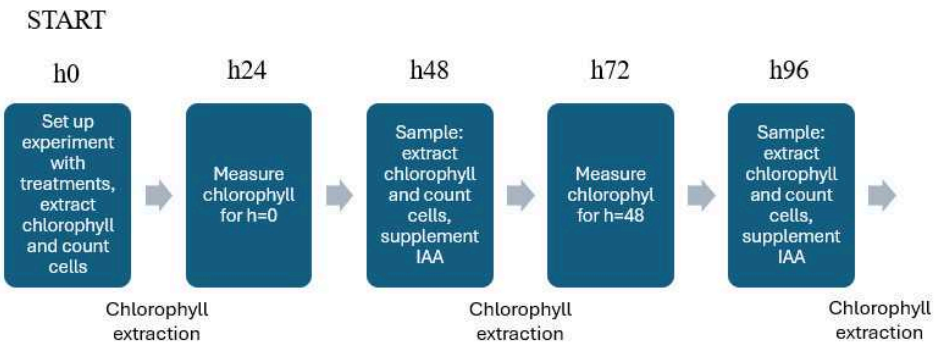
Guidelines



Suggested experimental workflow

Materials

MEADIA

FMAP (1 L)

15 g Marine Broth BD Difco™ Dehydrated Culture Media: Marine Broth 2216 **Thermo Fisher Scientific**

5 g Peptone

Peptone from casein (Tryptone) **Merck MilliporeSigma (Sigma-Aldrich)**

300 mL Filtered seawater

700 mL MilliQ water

Protocol materials

- Sodium metasilicate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**NA
- 1H-Indole-3-acetic acid, 98% **Thermo Scientific**
- 96-100% ethanol
- Guillard's (F/2) Marine Water Enrichment Solution 50 x **Merck MilliporeSigma (Sigma-Aldrich)**
- Peptone from casein (Tryptone) **Merck MilliporeSigma (Sigma-Aldrich)**
- BD Difco™ Dehydrated Culture Media: Marine Broth 2216 **Thermo Fisher Scientific**

Safety warnings

Refer to the Safety Data Sheets (SDS) for information on health and environmental hazards. Be sure to follow your institution's Health and Safety (HMS) guidelines, wear appropriate personal protective equipment (PPE), and adhere to all established safety protocols to minimize risks

Before start

This protocol outlines the axenic and paired cultivation of *Porosira glacialis* UiT201 and *Sulfitobacter marinus*, including IAA treatment; however, it can easily be adapted to other algal strains, bacteria isolates, and variable conditions to investigate diatom-bacteria growth dynamics. FMAP media was utilized as an all-purpose marine growth media in the creation of this protocol (see Materials for media composition), however it is highly recommended to establish optimal growth media for each bacterial strain. Results are assessed through cell density and chlorophyll a extraction.
Please note that your experimental design may vary. For this protocol, samples are measured and Indole-3-acetic acid (IAA) is supplemented every other day. Chlorophyll a and cell counts are recorded continuously throughout the experiment, which lasts until two repeated measurements are taken during the stationary phase. A suggested workflow is provided in the Guidelines section.

Experimental overview

Replicate	A1	A2	A3	B1	B2	B3	C1	C2	C3
Treatment	*UIT201*	*UIT201*	*UIT201*	*UIT201 + S. marinus*	*UIT201 + S. marinus*	*UIT201 + S. marinus*	*UIT201 + IAA*	*UIT201 + IAA*	*UIT201 + IAA*
Tot. Volume	*500 ml*								
Media	*F/2 8 ml/l (50x stock)+ Silicate 8 ml/l (3,5g/l stock)*								
Treatment Conc.	-			*50:1 bacteria:diatom ratio*			*50 nM*		
Treatment added	-			*Once at T=0*			*Every 2 days*		

Sampling and measurement scheme

Chl a	Every 2 days
Algae count	Every 2 days

Cultivation set-up

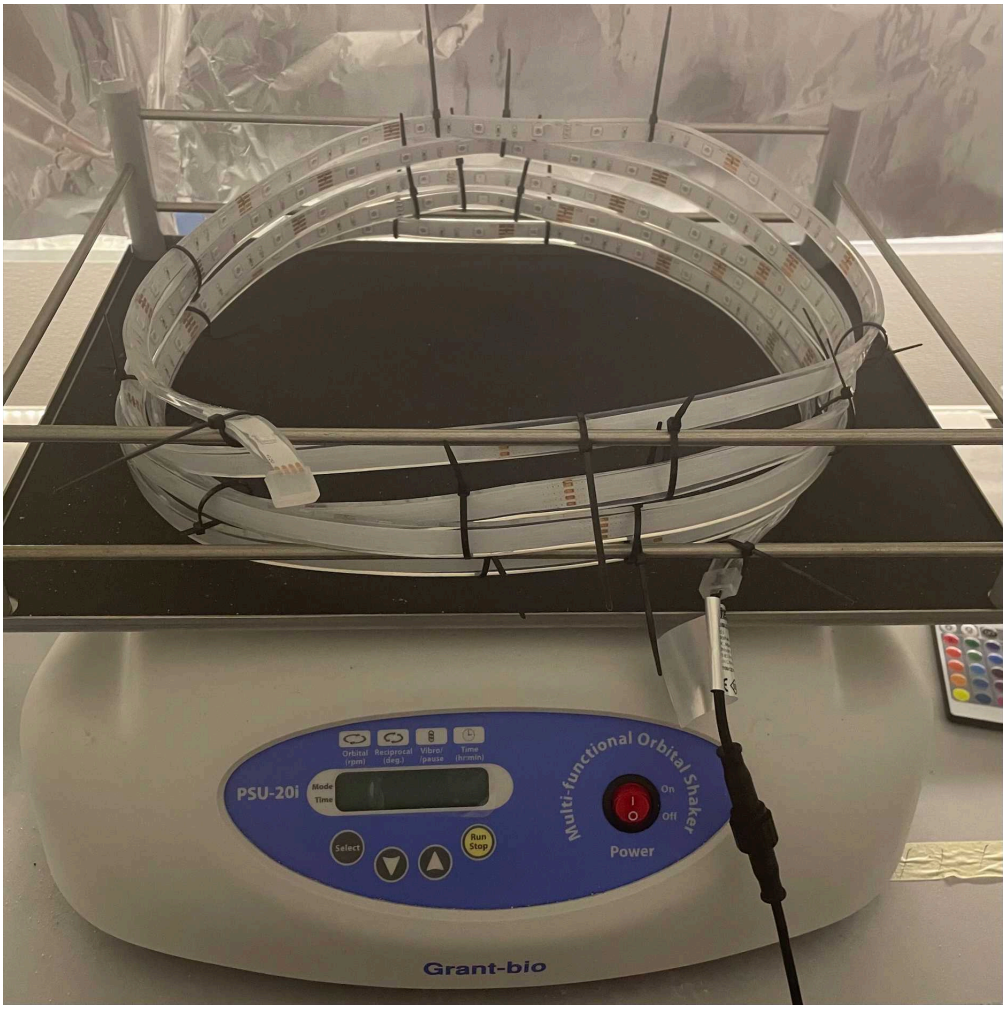
10m

1 Position a large orbital shaker in a 7 °C cold-room and arrange flexible 200 × 50W RGB LED strips along the shaker to provide a sufficient cultivation light source

5m

Equipment

PSU-20i Orbital Shaking Platform	NAME
Orbital Shaking Platform	TYPE
Grant bio	BRAND
NA	SKU
https://www.grantinstruments.com/products/psu-20i-orbital-shaking-platform	LINK



PSU-20i Orbital Shaking Platform with RGB light fixture

Day 1 (T= -2) - Treatment preparations

3h 20m

2 Inoculate bacteria isolates in 5 mL FMAP media in 15 mL culture tubes and shake Overnight

10m

3 Prepare a stock solution of [M] 0.25 millimolar (mM) 1H-Indole-3-acetic acid, 98% **Thermo Scientific** dissolved in Milli-Q water. Wrap the stock solution in tinfoil to prevent degradation, and place in a refrigerator for short term storage. For long term storage, consider storing at -20 °C .

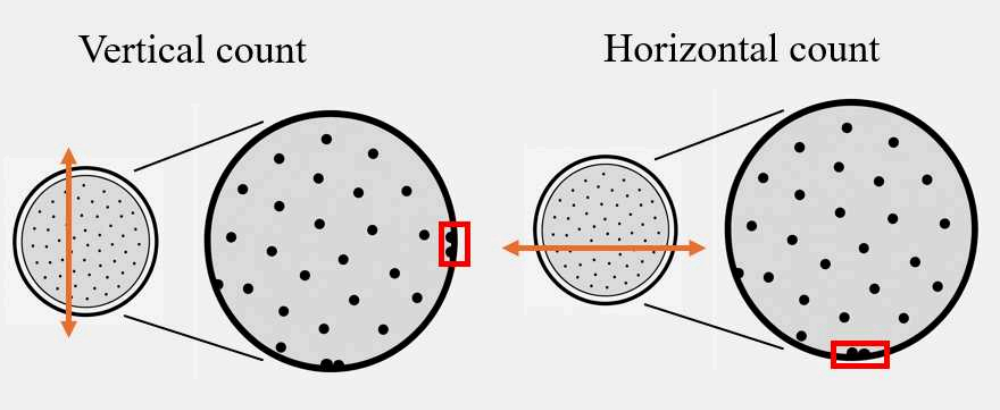
10m

4 The Utermöhl settling method (Edler, L., and Elbrächter, M., 2010) is utilized to count the stock algae culture: Sample 4 mL of stock diatom culture and fix 2 mL diatoms in duplicates with 7 µL Lugol's iodine solution in 2 mL cell culture wells for 1-2 hours in a 4 °C refrigerator.

2h

5 Once the samples have settled, observe it under a microscope. Diatom cells are identified and counted in several randomly selected fields of view to ensure a representative sample. If the diatom concentration is too large for an accurate count (>100), determine a dilution factor and proceed to dilute the culture. For each duplicate sample, count the diatom cells first horizontally, then vertically along an axis. For the horizontal count, exclude cells on the bottom edge of the microscopic view, and for the vertical count, exclude cells on the right edge. Repeat the process for the second duplicate sample. This results in four values which are averaged to calculate the concentration of cells per unit volume of the original water sample.

1h



Counting diatom cells using the Utermöhl method. Cells are counted horizontally and vertically, excluding those on the bottom and right edges (outlined in red boxes). This process is repeated for the second duplicate sample.

6 Aiming for a starting culture of 1-2 mill cells/L, dilute with Milli-Q to 1:3 of the starting algae concentration.

Note

This step decreases lag-phase, ensuring that a quick transition to log phase. Skipping this step could result in an extended lag-phase.

Day 2 (T= -1) - Treatment preparations

15m

7 Move the overnight bacteria inoculation tubes to a 7-degree cold room to acclimate. Leave Overnight for additional inoculation.

5m

8 Prepare a stock of [M] 3.5 g/L Sodium metasilicate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**NA in Milli-Q

10m

Day 3 (T=0) - Initiating cultivation

4h 35m

9 For determining stock diatom culture sample, utilize the Utermöhl settling method: 4 mL of stock diatom culture and fix 2 mL diatoms in duplicates with 7 µL Lugol's iodine solution in 2 mL cell culture wells for 1-2 hours.

2h

10 Prepare fresh media by adding [M] 8 mg/mL of Guillard's (F/2) Marine Water Enrichment Solution 50 x **Merck MilliporeSigma (Sigma-Aldrich)** and [M] 8 mg/mL of the stock sodium metasilicate to 5 L of filtered seawater*

* 5 L is sufficient for 9 (3 treatments * 3 biological replicates) replicates

5m

11 Filter the media through 0.22 µm sterile filters and fill 9 500 mL tissue culture flasks

30m

> Note
>
> Follow and do not exceed the recommended capacity of the filters

Equipment

Sterivex™ Pressure Filter	NAME
0.22 µm filter	TYPE
Millipore®	BRAND
00-00-0	SKU
https://www.sigmaaldrich.com/NO/en/product/mm/svgp010#product-documentation	LINK

Equipment

Masterflex L/S	NAME
Peristaltic Pump	TYPE
Masterflex	BRAND
NA	SKU
https://www.avantorsciences.com/us/en/product/39208424/masterflex-ls-digital-pump-system-with-gore-sta-pure-pfl-tubing-avantor	LINK

12 After 1-2 hours of fixation, utilize the Utermöhl method in step 5 to count the stock algae culture. 1h

13 Aiming for a starting culture of 1-2 mill cells/L, transfer diatoms to the 500 mL tissue culture flasks 10m

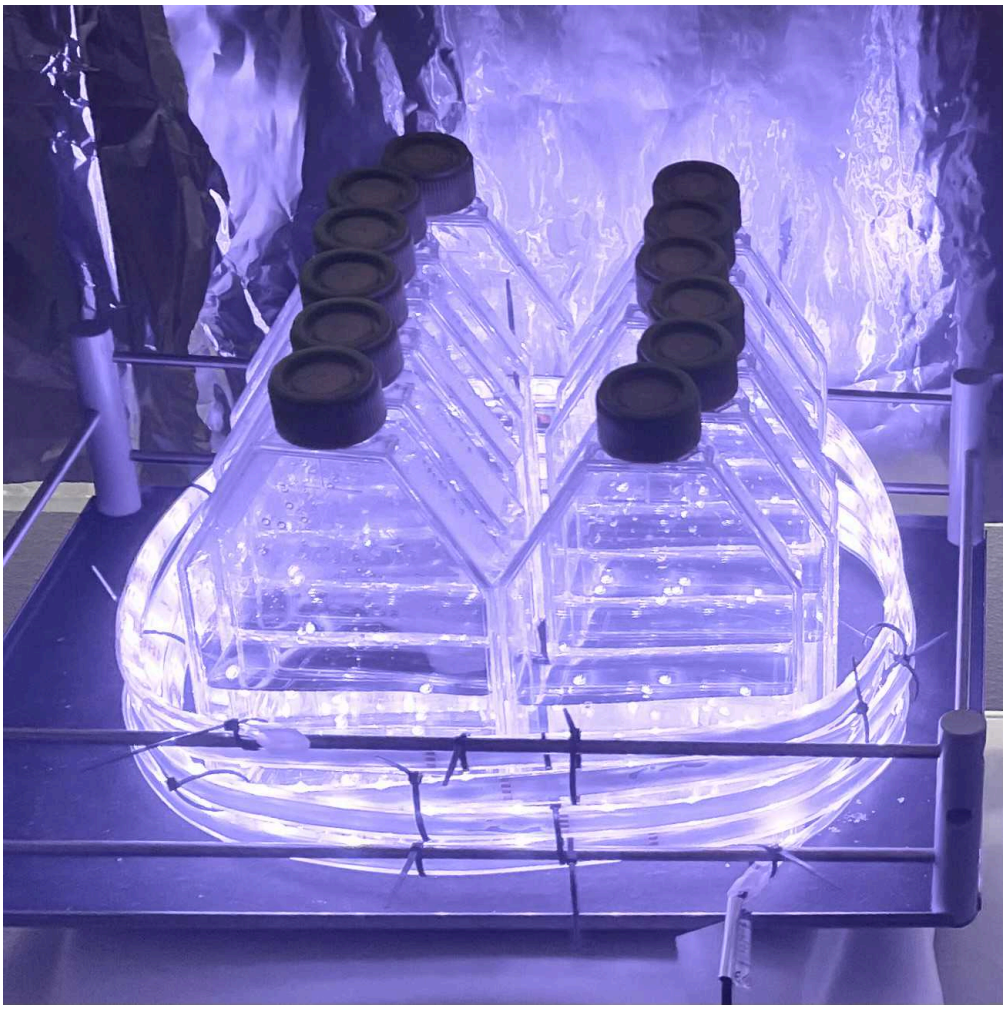
14 Determine bacteria isolate concentrations by measuring OD600. Aiming for a 50:1 bacteria:diatom ratio, proceed to pipette the appropriate amount to each of the bacteria treatment culture flasks 30m

15 From the [M] 0.25 millimolar (mM) 1H-Indole-3-acetic acid, 98% **Thermo Scientific** stock spike 100 µL of IAA to each of the IAA treatment culture flasks to achieve a final concentration of [M] 50 nanomolar (nM) . Immediately proceed to step 16. 5m

16 Turn over the culture flasks 10 times to homogenize the cultures and place the flasks on the orbital shaker in the 7 °C cold-room. Set the orbital shaker to 50 rpm, 7°C with 300 reciprocal motion for 12 seconds and vibration set to 2. 15m

Note

Measure light intensity ensuring consistent light intensity across all flasks. It is recommended to alternate the positioning of the bottles.



Culture bottle placement on orbital shaker in the 7 °C cold-room

Orbital shaker settings

Sampling for chlorophyll extraction and cell count

30m

17 Retrieve and extract 25 mL samples from the each of the culture bottles in 50 mL falcon tubes. To minimize incubation room downtime, immediately return the culture flasks to the orbital shaker. 30m

Note

For each measurement 4 mL is needed for cell counting and 20 mL for chlorophyll extraction. Extracting 25 mL of sample allows for some margin. As the diatom culture enters log phase, the samples need to be diluted for measurements and less extracted sample volume is needed.

Diatom cell count

2h

18 From each of the 25 mL samples, the utilize Utermöhl settling method is used to create duplicates count the cells (steps 4 and 5): pipette 2* 2 mL of culture to 2 mL cell culture wells and fix with 7 µL Lugol's iodine solution for 1-2 hours. Proceed to count the cells according to the previously described method. 2h

Chlorophyll extraction

15h

19 Assemble a vacuum pump with a fitted filtration system. From the remaining 21 mL of each sample culture, create 5 technical replicates by depositing 5 * 4 mL on 1.2 µm glass microfiber filter papers. Proceed to create a vacuum and filter the culture samples. 1h

Equipment

Grade GF/C Glass Microfiber Filter Papers	NAME
Microfiber Filter Papers	TYPE
Whatman	BRAND
NA	SKU
https://www.tischscientific.com/whatman/filter-papers-glass-microfiber-filters-1822-025	LINK
1.2um, 25mm Dia., Binder-Free	SPECIFICATIONS

Equipment

Chemical Duty Pump	NAME
Vacuum pump	TYPE
Merck Millipore	BRAND
NA	SKU
https://www.sigmaaldrich.com/NO/en/product/mm/wp6122050	LINK
220 V/50 Hz	SPECIFICATIONS

Equipment

1225 Sampling Manifold	NAME
Millipore	BRAND
NA	SKU
https://www.sigmaaldrich.com/NO/en/product/mm/xx2702550	LINK
15 mL process volume	SPECIFICATIONS

20 Transfer the glass microfiber filters to separate test tubes and add 5 mL 96-100% ethanol. For extraction, immediately cover the samples in tinfoil and place them in a 7 °C cold-room Overnight.

12h

21 After allowing overnight extraction, proceed to measure chlorophyll a on a fluorometer with excitation wavelength set to 430-450 nm (typically around 435 nm) and emission wavelength to 675-685 nm. A chlorophyll a factor of 0.00417 is used to convert absorbance readings into the concentration of chlorophyll a (Chla µg/L) in a sample.

2h

Equipment

TD-700 Laboratory Fluorometer	NAME
Fluorometer	TYPE
Turner Designs	BRAND
00-00-0	SKU
Discontinued	LINK

Protocol references

Edler, L., and Elbrächter, M. (2010). “The utermöhl method for quantitative phytoplankton analysis,” in *Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis*, Vol. 110, eds B. Karlson, C. Cusack, and E. Bresnan (Paris: Intergovernmental Oceanographic Commission Manual and guides), 13–20