Establishment of axenic sea-ice diatom cultures, modified from Jaeckisch et al. (2011)

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Abstract

Table 1. Antibiotic concentrations used for establishing axenic sea-ice diatom cultures.

Antibiotic	Final concentration (µg/ml)
Ampicillin	50
Gentamycin	15
Streptomycin	125
Chloramphenicol	10
Ciprofloxacin	10
Penicillin	100

References

Guillard RRL. 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH eds. *Culture of marine invertebrate animals*. New York: Plenum, 29-60.

Jaeckisch N, Yang I, Wohlrab S, Glöckner G, Kroymann J, Vogel H, Cembella A, John U. 2011. Comparative Genomic and Transcriptomic Characterization of the Toxigenic Marine Dinoflagellate Alexandrium ostenfeldii. *PLoS ONE* **6**(12): e28012.

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Protocol

Step 1.

Pre-treat an optically dense diatom culture with 3 min of vortex-mixing (< 8°C) and 30 sec of ultrasonication (80% in 5-s pulses) on ice to remove attached bacteria from diatoms and EPS aggregates.

Step 2.

Centrifuge cells at 1000 × g for 10 min (< 8°C) and wash the pellet twice with sterile F/2 medium (Guillard, 1975) to remove the majority of loose bacterial cells.

Step 3.

Add six antibiotics to the medium according to Table 1 (see abstract), and incubate for 5 days at –1°C and under light (20–45 µmol photons m^{-2} s^{-1}).

Step 4.

Centrifuge cells at 1000 × g for 10 min (< 8°C) wash pellet in fresh F/2 medium.

Step 5.
Pick < 10 cells to start the axenic cultures (pipetting or plate on F/2 agar to pick single colonies).

Step 6.
After recovery and growth (2 months), check for bacterial cells using DAPI staining and after agar plating on half-strength BD Difco™ Marine Agar 2216 (< 8°C), kept at seawater salinity using artificial seawater (411 mM NaCl, 9.39 mM KCl, 26.1 m M $MgCl_2 6H_2O$, 28.4 mM $MgSO_4 \cdot 7H_2O$, 5.01 mM TAPSO buffer).

Step 7.
If bacteria are still present, repeat protocol. Axenic cultures are usually established within the first or second attempt.