Growing open ocean ammonia-oxidizing archaea

Initial enrichment

Collect seawater in a large (preferably 500 mL or larger) polycarbonate bottles cleaned as described below.

Age the seawater in the dark at in situ temperatures for 6 months or more, without disturbing.

Add 10 uM NH4+ (final concentration) and monitor for NO2- or NO3- production. Repeat until enrichment has taken up a total of 50 uM NH4+ before transferring.

General considerations

Glassware and plasticware used for culturing should be used only for this purpose. Never use soaps or detergents on these items.

Handling time on the bench should be minimized. Light inhibition has not been fully tested.

Cultures do not like to be disturbed and appear to be sensitive to physical mixing. For example, if identical cultures are inoculated into new media and one culture is sampled daily and the other culture sampled weekly, the culture sampled weekly will reach exponential phase more quickly.

Cultures should be transferred as close to mid exponential phase as possible or the lag phase could be significant in the transferred culture (2-3 weeks). In practical terms, for a culture grown on 100 uM NH4+, transfer when the [NO2-] is 40-60 uM.

The optimal culture volume seems to be between 50 and 250 mL, but I have successfully grown 500 mL cultures. Right now the 50 mL cultures are being transferred every 10 days or so.

Do not throw out old cultures until two successful transfers have been completed (at least).

The quality of the seawater appears to be important. I suggest filtering into an acid washed 10 L carboy at sea or immediately on return to the lab. Store in the dark at 4ºC. Aged seawater seems to work better than 'fresh' seawater.

You may find it useful to measure the [NO3-] of the seawater you will use for media in case you suspect contamination of the cultures with NO2- oxidizers in the future. If the

water you chose to begin with was truly oligotrophic it should be low anyway.

Antibiotics, especially ampicillin, interfere with the commonly used phenol hypochlorite method of [NH4+] determination. If you want to monitor [NH4+], either leave out the antibiotics or use the OPA fluorescence method of Holmes et al. 1999.

Oligotrophic North Pacific (ONP) medium preparation

Recipe:
2 L low nutrient Pacific seawater
2 mL chelated trace element solution (recipe attached)
1 mL KH_2PO_4 solution (4g/L stock)
2 mL NH_4Cl stock solution (50-100 mM stock)
1 mL streptomycin solution (100 mg/mL frozen stock, optional)
1 mL ampicillin solution (100 mg/mL frozen stock, optional)
0.3 M K_2CO_3 as needed

Autoclave two 2 L screw-top glass media bottles. Filter 2 L of seawater into one of the bottles using a peristaltic pump with an attached 0.2 um cartridge filter (i.e Sterivex; optional, but I occasionally get a yellow-colored contaminant if I don't incorporate this extra step).

Add the remaining ingredients, screw the bottle closed, and invert several times to mix. Remove an aliquot and check the pH. Should be 7.6 - 7.8. Add K_2CO_3 if not. Do not raise pH above 8.0.

Attach a 0.2 um bottle top vacuum filter (Millipore Sterlitop PES) to the second media bottle. Filter the prepared media into the second bottle. Use immediately or store at 4ºC for up to 2 weeks.

Tests are ongoing with autoclaved media, but for now I would suggest the above media preparation.

Propagating cultures

All cultures are currently maintained in polycarbonate screwcap bottles (Nalgene) prepared according to the attached protocol.

Pour off MilliQ water from prepared bottles. Pipette or pour freshly made ONP media into bottles.

Transfer 10-20% final volume mid-exponential phase culture into fresh media. I typically do a 5 mL:45mL in the 60 mL bottles and 25mL:175mL in the 250mL bottles.

Incubate at 20ºC (CN25 or CN75) or 4-15ºC (CN150) in the dark. It is convenient to store them in the boxes the bottles come in.

Subsample with a 2mL serological pipette to measure initial [NO2-].

Cross your fingers.

Monitoring cultures

I monitor all the cultures every 5-10 days for NO2- production and transfer as necessary. I check the cultures using CARD-FISH every 1-2 months to monitor the % archaea. Should always be >75%.

Bottle preparation

Remove old markings with 100% ethanol.
Prepare 10% HCl with MilliQ water in a carboy.
Rinse bottles 5X with MilliQ water.
Fill one-quarter full with 10% HCl. Screw cap on, shake, and allow to sit at least overnight.
Pour HCl back into carboy.
Rinse bottles 5X with MilliQ water and fill with:
5 mL (60 mL bottles) or 25mL (250 mL bottles) of MilliQ water and loosely cap.
Arrange bottles around the edge of the carousel in microwave. Microwave 2 min (60 mL bottles) or 3-4 min (250 mL bottles). Make sure water boils.
Allow to cool slightly. Shake gently and microwave a second time.
Store bottles loosely capped in their original boxes.

Useful part numbers

60 mL polycarbonate screwcap bottles, Nalgene (2015-0060, Fisher 03-311-1K)
250 mL polycarbonate screwcap bottles, Nalgene (2015-0250, Fisher 03-311-1B)
0.2 um bottle top filters, Millipore Steritop (SCGPT05RE)
2 mL serological pipettes, BD (357507, case of 1000)
50 mL serological pipettes, BD (357550, pk of 100)

Trace Metal Solution (1000X)

EDTA-Na2 (14mM)
FeCl2-4H2O (7.25 mM)
ZnCl2 (0.5 mM)
MnCl2 (0.5 mM)
H3BO3 (1 mM)
CoCl2-6H2O (0.8 mM)
CuCl2-2H2O (0.1 mM)
NiCl2-6H2O (0.1 mM)
Na2MoO4-2H2O (0.15 mM)

Make up in 1L. Filter sterilize and store at room temp.

Reference

If this information was useful to you, please reference this paper:

Santoro, A.E. and K.L. Casciotti. 2011. Enrichment and characterization of ammonia-oxidizing archaea from the open ocean: phylogeny, physiology, and stable isotope fractionation. ISME Journal.

And email me to let me know if you have any luck!
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