Isotopic labeling of freshwater mixotrophic algae using isotopic labeled inorganic nitrogen and carbon

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Abstract

This protocol aims to achieve an isotopic labeling of mixotrophic algae using $^{15}NH_4Cl$ and ^{13}C-bicarbonate. It was developped to be used with the mixorophic chrysophyte *Ochromonas* to asses the sources of carbon and nitrogen of this algae when growing mixotrophically . Sources of nitrogen and carbon in the media used were reduced to 1 inorganic source (NH_4Cl for nitrogen and bicarbonate for carbon) and 1 organic source (heat-killed bacteria); media used for *Ochromonas* growth was modified accordingly to reflect this.

Downstream analysis of isotopic labeled Ochromonas include bulk IRMS measurements or nanoSIMS measurements.

Citation: Ramon Terrado Isotopic labeling of freshwater mixotrophic algae using isotopic labeled inorganic nitrogen and carbon. **protocols.io**
dx.doi.org/10.17504/protocols.io.ewmbfc6
Published: 18 May 2016

Protocol

Step 1.

Prepare modified DY-V media with labeled substrates

Media used for Ochromonas growth was a modified version of DY-V media. Modifications were:

- No MES was used, as it can be used as a source of carbon (bicarbonate and heat-killed bacteria are the only sources of carbon used in this media)
- No Na_2SiO_3 was used (*Ochromonas* does not need silica)
- No $NaNO_3$ (ammonium and heat-killed bacteria were the only sources of nitrogen used in this media)
- Sodium bicarbonate added at a final concentration of 95 µM. Bicarbonate addition is done before algal inoculum by 0.2 µm filtration of a stock solution (do not autoclave).

In order to ensure isotopic labeling of the mixotrophic algae, 50% of the ammonium chloride added to the media is ammonium-^{15}N chloride (98% atom ^{15}N) and all bicarbonate added to the media is ^{13}C-bicarbonate.

NOTE: depending on the degree of labeling that wants to be achieved, the % of labeled ammonium and bicarbonate can be modified.

LINK: https://ncma.bigelow.org/media/pdf/NCMA-algal-medium-DY-V.pdf

Step 2.

Media inoculation

Add heat-killed bacteria (HKB) to the media (HKB were obtained following this protocol).

Add an inoculum of the mixotrophic algae (volume of inoculum should be low as to avoid dilution of the isotopic label and carry over of nutrients).

NOTE: as an example, values commonly used were $5x10^3$ Ochromonas mL^{-1} and $5x10^7$ HKB mL^{-1} as starting concentrations for the cultures.

Step 3.

Mixotrophic growth of the algae

Let the algae grow so it incorporates the isotopic signature in its biomass. Track the algae growth through microscopy (live samples and/or fixed samples) and the decline of heat-killed bacteria (fixed samples and staining with DAPI to assess HKB concentrations through epifluorescence microscopy.

Step 4.

Sampling for assessment of algal isotopic signature

Ochromonas was allowed to grow for 2-3 generations before sampling for isotopic signature. Two kind of samples can be collected:

- Bulk measurements: filter 30-50 mL of the cultures onto pre-combusted glass fiber filters and dry at 60 ºC over night to stop all biological activity. Afterwards, filters can be stored in glass

vials at room temperature. Further processing of the sample included an acidification step with HCl to remove inorganic carbon and the C- and N- isotopic composition of the sample was determined by an isotope rato mas spectrometer (IRMS).

- Cell-specific measurements: collect 2 mL of sample and fix with 2X EM-grade glutaraldehyde. Sample can be stored in the fridge at 4 ºC. Further processing of the sample involves the deposition of cells onto sylicon wafers, washwith MQ-water and drye; map cells on the waffer using microscopy and analysis of single cells using a Cameca NanoSIMS 50 instrument (NOTE: actual manipulation of the machine will be done by an expert user).