Active heterotrophic bacterial assemblages involved in inorganic carbon fixation in the Pacific Northwest coastal margin

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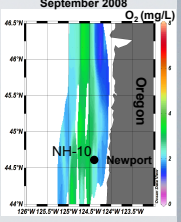
1. Introduction

- Carbon utilization by heterotrophic bacteria is attributed largely to oxidation of organic carbon coupled to respiration. Heterotrophs consume oxygen produced by autotrophs while fixing dissolved organic carbon (DOC) through glycolysis and the citric acid cycle.
- Carbon cycling in hypoxic water is more complex, however, in that autotrophs continue to fix dissolved inorganic carbon (DIC) as DOC becomes depleted due to elevated microbial activity.
- The goal of this project is to identify the organisms and processes in the hypoxic zone that are involved in inorganic carbon (DIC) utilization. The findings will shed light on the influence of marine hypoxia has on biogeochemical cycling of carbon.
- Our study, which employs Stable Isotope Probing (SIP) of inorganic carbon fixation, has uncovered evidence of a process if inorganic carbon utilization in the hypoxic zone that operates in heterotrophic bacterial taxa.

Dissolved Oxygen Concentration @ 80m September 2008



2. Identifying microorganisms active in inorganic carbon fixation via Stable Isotope Probing (SIP)

A. N_2

Bottles containing sea water were purged with nitrogen to reduce the oxygen concentration to in situ levels.

B. 2mM addition of $NaH^{13}CO_3$

Bottles were incubated at 7°C for 3 hours

Fractionated DNA illuminated by EtBr during SIP

C. DNA was extracted from cells that were harvested by filtration. The DNA was applied to a CsCl density gradient for fractionation.

DNA from organisms that utilize $NaH^{13}CO_3$ will incorporate ^{13}C and can be separated from ^{12}C DNA through centrifugation.

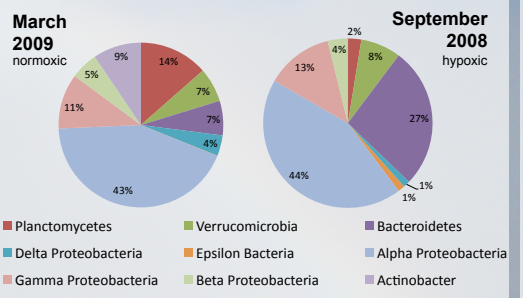
D. ^{12}C and ^{13}C bands of DNA were then recovered, and 16S rDNA was amplified with general bacterial primers 27f and1492r. Cloning was conducted at the Washington University Genome Sequencing Center.

E. Sequences were cleaned and subjected to phylogenetic analysis utilizing ARB.

An increase in bacterial heterotrophic diversity involved in the assimilation of DIC may reflect an adaptive response to a depletion of complex organic carbon sources during a hypoxic event.

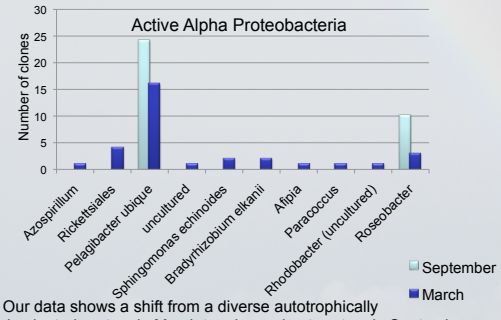
3. SIP identifies a seasonal shift in active bacterial populations

16S clone data from active ^{13}C SIP fractions

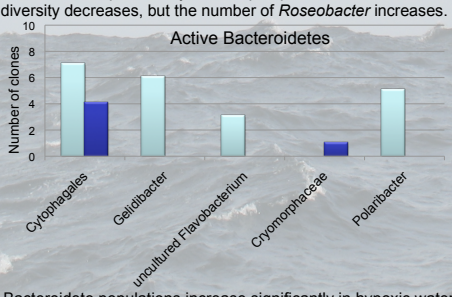


Many of the identified classes include heterotrophic bacteria.

4. Hypoxic conditions may influence bacterially mediated carbon fixation



- Our data shows a shift from a diverse autotrophically dominated system in March to a less robust system in September.
- This is evident particularly in the Alpha proteobacteria where the diversity decreases, but the number of *Roseobacter* increases.



- Bacteroidete populations increase significantly in hypoxic waters and are active in DIC assimilation.
  - These organisms are the dominant heterotrophic population.
  - The increase in diversity within this class may indicate a competitive advantage over other organisms under low oxygen conditions.
- Other heterotrophic organisms active in the system include Verrucomicrobia (primarily represented by *Prosthecobacter*) and Planctomycetes (strongly represented by *Pirellula).*

5. Anaplerotic carbon fixation in hypoxic waters

- The observed DIC fixation is likely caused by anaplerotic reactions mediated by phosphoenolpyruvate carboxylase (PEPC) and pyruvate carboxylase.
- These reactions are very well known to occur in the environment. Roslev, et al. 2004, suggested that 3 to 8% of the organic carbon in some heterotrophic bacteria originates from CO_2 incorporated by carboxylation reactions.
- The presence of bacterial PEPC has been confirmed in both September 2008 and March 2009 environmental samples through PCR amplification followed by cloning.

We hypothesize that as good quality DOM is depleted under hypoxic bloom conditions the identified heterotrophic organisms supplement their TCA cycle via anaplerotic carbon fixation.

- Shifts towards increased anaplerotic carbon fixation have been previously observed in algae (Guy, Vanlerberghe, & Turpin,1989).

6. *Polaribacter irgensii* as a model organism for anaplerotic carbon fixation in hypoxic waters

- To test our hypothesis regarding the advantage of anaplerotic carbon fixation in hypoxic waters we will utilize *P. irgensii.*
- *P. irgensii* is present in our 16S clone library of the active ^{13}C SIP fraction from our September 2008 hypoxic sample .
- Anaplerotic CO_2 fixation pathways mediated by pyruvate carboxylase and PEPC have been identified in *P. irgensii* through genome sequencing.

Oxic
P. Irgensii will be grown on M9 media supplemented with malate.

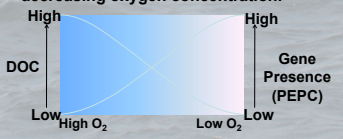
Or

Hypoxic/Anoxic
P. Irgensii will be grown on M9 media supplemented with glucose.

The preferred carbon source will gradually be reduced and replaced with DOM typically produced by algae (glycolate, taurine, and DMSP).

At various points in the growth curve RNA will be extracted and qRT-PCR utilized to determine PEPC levels.

We hypothesize that PEPC gene copy numbers increase as DOC quality decreases. This may be exacerbated with decreasing oxygen concentration.



7. Summary

- Heterotrophic bacteria were implicated in the assimilation of dissolved inorganic carbon (DIC), which may reflect an adaptive response to a depletion of complex organic carbon sources following a hypoxic event.

The persistence of active bacterial heterotrophic populations assimilating CO_2 and the decrease in autotrophic bacterial diversity suggests that heterotrophic bacteria able to assimilate DIC may have an advantage for survival during hypoxic events.