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 nitrogen fixation.

• Our study, which employs Stable Isotope Probing (SIP) of inorganic carbon fixation, has uncovered evidence of a process in which inorganic carbon utilization in the hypoxic zone that operates in heterotrophic bacterial taxa.

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

A. N2

Bottles containing sea water were purged with nitrogen to in situ levels.

B. 2mM addition of NaH13CO3

Bottles were incubated at 7°C for 3 hours.

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

D. 13C and 12C bands of DNA were then recovered, and 16S rDNA was amplified with general bacterial primers 27f and 1492r.

Cloning was conducted at the Washington University Genome Sequencing Center. 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

March 2009

Gamma-Proteobacteria

Beta-Proteobacteria

Siphonomonas echinoides

April 1

March 2009

April 1

Alpha-Proteobacteria

Beta-Proteobacteria

Epsilon-Proteobacteria

Curvibacter

Two SIP identifications from our March 2009 sample.

4. Hypoxic conditions may influence bacteriologically 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.

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. Roselius, et al, 2004, suggested that 3 to 8% of the organic carbon in some heterotrophic bacteria originates from CO2 incorporated by carboxylation reactions.

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.

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 13C SIP fraction from our September 2008 hypoxic sample.

• Anaplerotic CO2 fixation pathways mediated by pyruvate carboxylase and PEPC have been identified in P. irgensii through genome sequencing.

We hypothesize that PEPC gene copy numbers increase as DOM quality decreases. This may be exaggerated by 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 decrease in autotrophic bacterial diversity suggests that heterotrophic bacteria able to assimilate DIC may have an advantage for survival during hypoxic events.