Molecular approaches for *in situ* identification of nitrate utilization by marine bacteria and phytoplankton

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Abstract

The flux of nitrogen and carbon in ocean margins are intertwined since primary productivity is often limited by the availability of nitrogen. A key aspect of nitrogen cycling is the flux and consumption of nitrate and ammonium, often referred to as "new" and "regenerated" sources of nitrogen for biological productivity. The objective of this proposal is to develop a molecular biological approach to studies of nitrate flux in marine communities. The results will provide new techniques for examining the competition and interactions between phytoplankton and bacterioplankton during the assimilation of nitrate and ammonium in marine environments.

We propose to capitalize on advancements in molecular biology and the availability of a small but significant genetic database for the genes that encode assimilatory nitrate reductase genes. Using this data, group-specific PCR primers and probes will be constructed to characterize and quantify the expression of nitrate reductase genes from bacteria and phytoplankton. Previously, the relative roles of bacteria and phytoplankton in nitrogen assimilation could only be addressed with indirect approaches such as size-fractionation. The application of this approach, in conjunction with conventional tracer strategies, will provide new information on the importance of bacterial and phytoplankton nitrogen uptake in regulating carbon and nitrogen flux in ocean margins. The trophic implications of bacterial and phytoplankton utilization of nitrogen will be investigated in experimental manipulations. These experiments will use molecular probe assays to determine whether selective grazing on nitrate utilizing organisms occurs.

The results of this study will provide important new tools for assaying the roles of different microorganisms in nitrogen flux, and will be integrated with conventional tracer techniques to provide new information on nitrogen limitation in the marine environment, and its regulation of carbon production in ocean margins.