

Molecular approaches for *in situ* study of nitrate utilization by marine bacteria.

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Abstract

Traditionally, the importance of inorganic nitrogen (N) for the nutrition and growth of marine phytoplankton has been recognized, while inorganic N utilization by bacteria has received less attention. Likewise, organic N has been thought to be important for heterotrophic organisms but not for phytoplankton. However, accumulating evidence suggests that bacteria compete with phytoplankton for nitrate (NO₃) and other N species. The consequences of this competition may have a profound effect on the flux of N, and therefore carbon (C), in ocean margins. Because it has been difficult to differentiate between N uptake by heterotrophic bacterioplankton versus autotrophic phytoplankton, the processes that control N utilization, and the consequences of these competitive interactions, have traditionally been difficult to study. Significant bacterial utilization of DIN may have a profound effect on the flux of N and C in the water column because sinks for dissolved N that do not incorporate inorganic C represent mechanisms that reduce the atmospheric CO₂ drawdown via the “biological pump” and limit the flux of POC from the euphotic zone.

Since 1998 with the support of the DOE Biotechnology Investigations – Ocean Margins Program (BI-OMP), we have developed a tool kit of molecular methods (PCR, RT-PCR, Q-PCR, QRT-PCR, and TRFLP) that allow selective isolation, characterization, and study of the diversity and genetic expression (mRNA) of the structural gene responsible for the assimilation of NO₃ by heterotrophic bacteria (*nasA*). - To date, our studies have revealed that bacteria capable of assimilating NO₃ are ubiquitous in marine waters, that the *nasA* gene is expressed in these environments, that heterotrophic bacteria can account for a significant fraction of total DIN uptake in different ocean margin systems, that the expression of *nasA* is differentially regulated in genetically distinct NO₃ assimilating bacteria, and that the best predictors of *nasA* gene expression are either NO₃ concentration or NO₃ uptake rates (Allen et al. 1999; Allen et al. in press; Booth et al. in prep). These studies provide convincing evidence of the importance of bacterial utilization of NO₃, insight into controlling processes, and provide a rich dataset that can be used to develop linked C and N modeling components necessary to evaluate the significance of bacterial DIN utilization to global C cycling. Furthermore, as a result of prior BI-OMP funding we have made exciting strides towards institutionalizing a research and education based collaboration between the Skidaway Institute of Oceanography (SkIO) and Savannah State University (SSU), an historically black university within the University System of Georgia with undergraduate and graduate programs in marine science. The long-term goals of these collaborations are to increase the capacity for marine biotechnology research and to increase representation of minorities in marine and environmental sciences.

Based on the success of our previous BI-OMP activities, we request renewed funding to continue studies of the role of heterotrophic bacteria utilization of NO₃, to develop new molecular tools for the study of urea utilization by bacteria, and to continue collaborative activities with SSU and another undergraduate institution, Roanoke College. A significant new objective of the proposed project is to quantify the importance of bacterial assimilation of NO₃ on ocean C cycling via direct effects on CO₂ fluxes and indirect trophic structure effects. These goals will be accomplished by the development of microbial sub-models that explicitly represent competitive interactions between autotrophs and heterotrophs for DIN and incorporation of these components into published ocean carbon models (OCMs).

The results of this study will continue to provide new molecular and biogeochemical approaches for studying questions pertaining to the utilization of N by bacteria and the importance of competition between heterotrophs and autotrophs for N resources to C cycling in ocean margin environments. The program will also support a framework for the continuation and expansion of a partnership between SkIO and SSU. There is no doubt that if this program is successful, it will serve as an important and sustainable mechanism to increase the number of under represented scientist (African Americans) in the field of marine science.

