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

Funded by DOE. 12/00 to 11/03.

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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 historically received less attention. However, accumulating evidence suggests that bacteria compete with phytoplankton for nitrate (NO_3^-) and that heterotrophic bacteria may have a profound effect on the flux of N, and therefore carbon (C), in ocean margins. Unfortunately, the processes that regulate the assimilation of NO_3^- by bacteria are not well understood, because it is difficult to differentiate between bacterial and phytoplankton N uptake using conventional tracer techniques. This is critical because sinks for dissolved N that do not incorporate inorganic C represent mechanisms which reduce the drawdown of CO_2 via the “biological pump”.

During the past 2.5 years, with the support of the DOE Biotechnology Investigations – Ocean Margins Program, we have developed molecular tools (PCR and RT-PCR primer sets) that allow us to selectively isolate, characterize, and study the diversity and genetic expression (mRNA) of the structural gene responsible for the assimilation of NO_3^- by heterotrophic bacteria (*nas*A). To date, our studies have revealed that bacteria capable of assimilating NO_3^- are ubiquitous in marine waters, and that the expression of *nas*A can be regulated in model organisms by the concentration of NH_4^+.

Based on the success of our previous BI-OMP program, we plan to continue the development of molecular tools for studying the role of heterotrophic bacterial utilization of NO_3^- and to use these tools in conjunction with new molecular methods (T-RFLP and quantitative PCR) to investigate the factors regulating bacterial NO_3^- utilization in diverse ocean margin environments, the nature of the competition between heterotrophic bacteria and phytoplankton for NO_3^-, and the functional importance of the existence of diverse bacterial populations capable of utilizing NO_3^-. These studies will build on the collaborations that are currently in place. The results of this study will continue to provide new molecular methods to address questions relative to the utilization of NO_3^- by bacteria and the underlying mechanisms that control NO_3^- flux into bacteria.