# **APPENDIX VIII**

# MOLECULAR CHARACTERIZATION AND REGULATION OF AMMONIA ASSIMILATION IN CHEMOAUTOTROPHIC PROKARYOTE-EUKARYOTE SYMBIOSES

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# **Objectives**

- Determine pathways and mechanisms of organic nitrogen synthesis by deep-sea hydrothermal vent communities
- Assess the influence of geochemical processes on the capacity for organic nitrogen synthesis.

## Approach

• Characterize nitrogen assimilation pathways in vent symbioses by enzyme activity measurements and DNA and immunoblot analyses.

## Results

- Activities of key enzymes (glutamine synthetase (GS), glutamate dehydrogenase (GDH), and nitrate reductase (NR)) involved in ammonia and nitrate assimilation by free-living bacteria and other autotrophic organisms are present in vent symbioses.
- Differences between species in capacity for symbiont-based sulfur-oxidation and
- carbon fixation correlate with activities of GS, GDH, and NR.
- Based on Southern hybridizations and immunological detection, symbiont GS of all vent symbioses tested is a dodecameric type I form found in many species of freeliving bacteria.
- The capacity for nitrogen assimilation in the vent tubeworm, *Riftia pachyptila*, *is* affected strongly by proximity to active venting. Worms collected at a low temperature site exhibited a drastic reduction in NR activity, GS activity in host tissue, and symbiont GS protein.