

## Molecular techniques for quantifying microbial population structure and activity in redox-stratified environments

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Recent advances in the molecular tools now available to microbial ecologists has dramatically changed the way in which we examine the make-up and dynamics of microbial assemblages in natural water systems. The molecular tools permit microbial ecologists to directly access ecologically relevant information in the form of phylogenetic- or phenotypic-specific macromolecules. Such macromolecules can be extracted from the environment, and thereby also avoid the biases associated with traditional cultivation techniques. Typically, only a small fraction (<1%) of the natural microbial population can be cultivated by standard techniques. The molecular techniques allow microbial ecologists to describe and quantify the relative abundance of specific phylogenotypes in complex microbial assemblages. Such an approach has resulted in the identification of a bewildering array of as yet unculturable microorganisms, many of which belong to the domain *Archaea*. Specific metabolic properties (e.g. metabolic pathways for energy generation or carbon dissimilation) can be inferred from analysis of the retrieved phylogenetic-specific markers. Nucleic acid sequence analysis of genes located proximal to the phylogenetic-specific markers (chromosome walking of environmental DNA retrieved directly from natural biomass) provide a means for confirming the metabolic potential of mixed microbial populations. Whole cells retrieved from the environment are also amenable to molecular-based hybridization techniques when combined with epifluorescent microscopy.

We have employed a variety of the cultivation- and molecular-based approaches to determine the

spatial and temporal variability in the microbial community structure of the redox-stratified salt marsh sediments of Sapelo Island, GA, a barrier island located of the southeast coast of the United States. Sediment samples have been collected on a seasonal basis from three salt marsh sites including a tidal creek bank, and within a zone of either tall or short spartina. Traditional cultivation methods have resulted in the identification of several metabolic types of microorganisms, including those capable of reducing oxygen, nitrate, nitrite, manganese-oxides, iron-oxides, uranyl carbonate, selenate, selenite, and sulphate as sole terminal electron acceptor. The relative abundance and depth profiles of this set of metabolically-distinct microorganisms is currently being compared to the profiles obtained from deployment of phylogenetic-specific molecular probes. The microbial signals are also being compared to those signals obtained from a parallel set of geochemical measurements of the corresponding porewater and solid phase sediments. Together, this data has provided information on the spatial and temporal variability in the structure and activity of the natural microbial population that inhabits the redox-stratified salt marsh sediment.

Future work on the molecular microbial ecology of natural water systems will ultimately be directed toward the development of phenotype- (or function-) specific molecular tools. As such tools become available, an even better understanding of the structure and activity of naturally-occurring microbial populations will be achieved.