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Molecular Diversity of δ-Proteobacterial Sulfate Reducers in Salt Marsh Sediment

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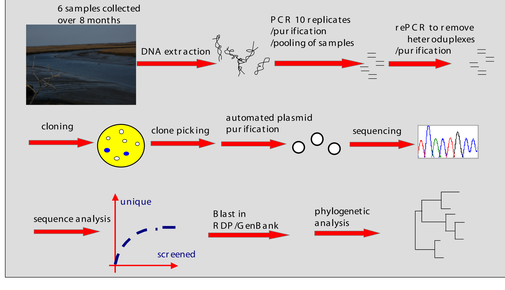
ABSTRACT

Sulfate-reducing bacteria (SRB) dominate the mineralization of organic matter in salt marsh environments. In these environments, SRB are believed to be represented by two phylogenetically distinct groups, Gram-positive and δ-Proteobacteria. We assessed the 16S rRNA diversity of δ-Proteobacteria SRB in salt marsh sediments of Plum Island Sound, MA by PCR, cloning and sequencing. Six samples taken over the course of a year were extracted to ensure representation of shifts in SRB community tied to seasonal growth cycles of the marsh grass *Spartina*. A clone library was constructed using 385F (selective for SRB δ-Proteobacteria) and 1492R (general bacteria) primers. Phylogenetic relationships were detected by the neighbor-joining method, whereas overall sampling efficiency and diversity were estimated using rarefaction analysis and the Chao-1 richness estimator. The primers also amplified other groups, such as ε-Proteobacteria, Firmicutes, and Cytophaga/Flexibacter, since they were chosen to match all SRB δ -Proteobacteria. Out of more than 1,000 analyzed sequences, over 50% belonged to members of δ-Proteobacteria. Among the δ-Proteobacteria, most of the ribotypes (unique sequences) were associated with members of the *Desulfobacteriaceae* (*Desulfosarcina*, *Desulfobacterium* and *Desulfonema*) and *Desulfobulbaceae* (*Desulfobulbus*, *Desulfofustis* and *Desulfotalea*) group. Sequences from the *Desulfomicrobiaceae* and *Desulfoarculaceae* groups were also observed. Over two thirds of the δ-Proteobacteria ribotypes were most similar to *Desulfosarcina* and *Desulfobacterium*, suggesting that complete oxidizers dominate this environment. Previously, it has been suggested that incomplete oxidizers dominate the marsh community since environmental acetate production was estimated to account for only 10% of sulfate reduction in salt marshes. Furthermore, no close relatives of *Desulfovibrio* were detected, despite their sequence match to the amplification primers and previous reports suggesting their presence in marsh sediments.

BACKGROUND

- **SALT MARSHES**
  - Land-sea interface; buffer zone
  - Areas of high primary productivity (460-3200 $gCm^{-2}y^{-1}$).
  - Sulfate-reduction is a dominant carbon remineralization process (over 50% of C_{org} is oxidized via sulfate reduction)
- **SULFATE REDUCING BACTERIA**
  - Anaerobic heterotrophs
  - 2 groups important in sediments:
    - Gram negative mesophilic (δ Proteobacterial) SRB
    - Gram positive SRB
- **COMMUNITY STRUCTURE OF SRB IN SEDIMENTS**
  - Cold sediments: *Desulfosarcina-Desulfococcus*
  - Intertidal surface sediments: *Desulfovibrio*, *Desulfosarcina-Desulfococcus-Desulfofrigus* group
  - Salt marsh sediments: *Desulfonema-Desulfococcus-Desulfosarcina* group

OUTLINE OF EXPERIMENTAL SETUP



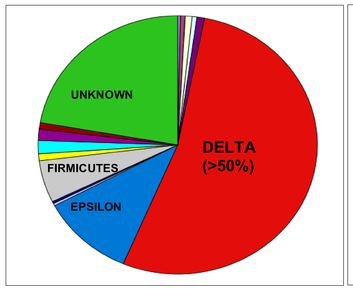


Figure 1. DISTRIBUTION OF SEQUENCED CLONES. Primers were chosen to not discriminate against any δ-Proteobacterial SRB. Thus a considerable fraction of clones (~50%) stem from other phylogenetic groups

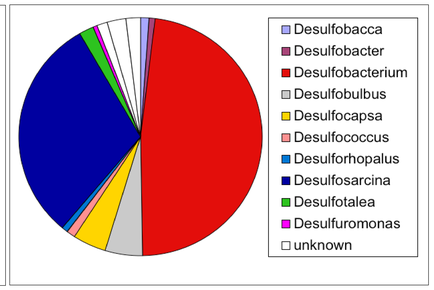


Figure 2. DISTRIBUTION OF SRB SEQUENCES. Over 80% of the sequences belonged to *Desulfobacteriaceae* which are completely oxidizing SRB (oxidize C_{org} to CO_2). About 12% of sequences belong to *Desulfobulbaceae*, incomplete oxidizers.

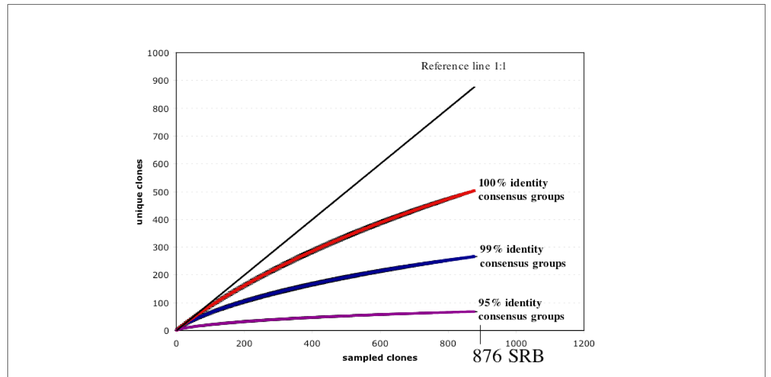


Figure 3. DIVERSITY ESTIMATE OF DIFFERENT CONSENSUS GROUPS BY RAREFACTION ANALYSIS. The estimated diversity was much higher than expected, indicating that even the extensive sampling of over 1,000 SRB sequences did not account for the total diversity as extrapolated by rarefaction analysis (method based on resampling without replacement). A large fraction of this diversity can be attributed to the ribotypes with the >99% identity. As indicated by Chao-1 richness estimator, when sequences grouped into 100% and 99% identity consensus groups there were 971 and 458 unique ribotypes, respectively.

Figure 4. SATELITE IMAGE OF PLUM ISLAND SOUND ESTUARY. The sampling site, Law's point on the Rowley River, is marked with a yellow circle. Triplicate core samples were collected monthly from the monophyletic stand of tall form *Spartina alterniflora* (marsh cord grass) rhizosphere during the plant's growing season (March 1999 through October 2000).

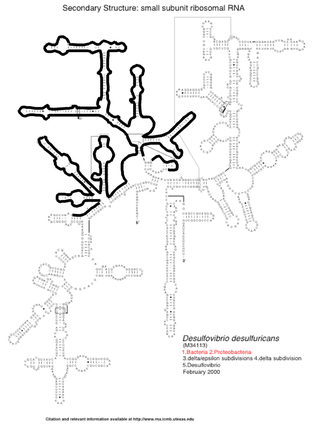


Figure 5. SECONDARY STRUCTURE OF 16S rRNA. Primer (385F, selective for primer for δ-Proteobacterial SRB and 907R (general bacteria) regions are marked in red and a sequenced region is marked in blue.

Figure 6. PHYLOGENETIC TREE OF -PROTEOBACTERIAL SRB SEQUENCES. Shown in blue are representative clone sequences of 99% identity consensus groups. Reference sequences are shown in black. The tree was constructed using Neighbor-joining algorithm in ARB.

RESULTS

- Close to 2000 clones were sequenced, out of which 876 were SRB-like clones as determined by blast against GenBank and RDP 16S rDNA database.
- A large fraction of the ribotype diversity can be attributed to the ribotypes belonging to >99% identity sequence consensus groups.
- Most of the sequences (> 80%) belonged to completely oxidizing SRB (*Desulfobacteriaceae*) with the majority of sequences matching closely with *Desulfosarcina*, *Desulfonema* and *Desulfobacterium*.
- Some monophyletic ribotypes did not fall within known SRB groups suggesting new lineages.
- A substantial fraction of 16S rRNA sequences belonging to δ–Proteobacterial group is closely related to sequences isolated from other marsh environments as well as from permanently cold, Arctic marine sediments (uncultured Sva0405 and Sva00863).
- Only 5 sequences distantly related to *Desulfovibrio* group were observed despite the ability of *Desulfovibrio* species to oxidize the very abundant substrate - ethanol.

CONCLUSIONS

- Oligonucleotide primers specific for δ-Proteobacterial group were used in PCR to avoid missing some low abundant SRB organisms.
- The high overall diversity in this clone library and rarefaction analysis suggests that we have sampled a substantial fraction of the SRB community.
- The dominance of completely oxidizing SRB suggests that the variety of plant exudates regulate their abundance and diversity.

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