

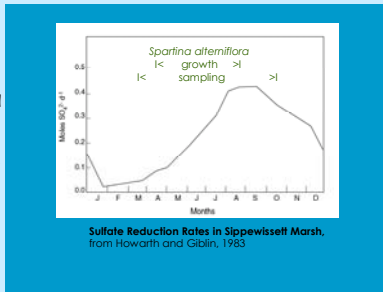
Diversity of Sulfate Reducing Prokaryotes in a Salt Marsh: Analysis by Sequencing

Dissimilatory Sulfite Reductase (*dsr*) Genes

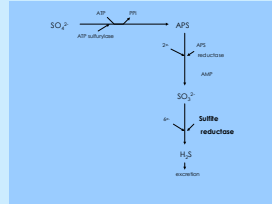
Michele Bahr¹ Byron Crump² and Andreas Teske³

¹ Marine Biological Laboratory, Woods Hole, Ma ² Univ. Maryland Center for Environmental Science ³ Univ. North Carolina, Chapel Hill

Sulfate reduction is the dominant remineralization pathway in salt marshes and surpasses aerobic respiration, denitrification, and metal reduction. Particularly high sulfate reduction rates have been found associated with the rhizosphere of the tall form of *Spartina alterniflora*, with distinct annual cycles. Rates at tall *S. alterniflora* sites increase up to fivefold between June and early August, when plants elongate aboveground and concurrently produce significant amounts of new rhizome material belowground which leaks dissolved organic compounds (Hines et al. 1989).



A key enzyme of dissimilatory sulfate reduction, **dissimilatory sulfite reductase (*dsr*)** catalyses the reduction of sulfite to sulfide, an essential step in the anaerobic sulfate respiration pathway.



In this study we investigated the diversity in the **Plum Island** salt marsh rhizosphere using full-length sequences of PCR-amplified *dsrAB* gene fragments. For analysis of partial *dsrAB* sequences, the alignment was limited to sequence regions corresponding to relevant environmental *dsrAB* sequences

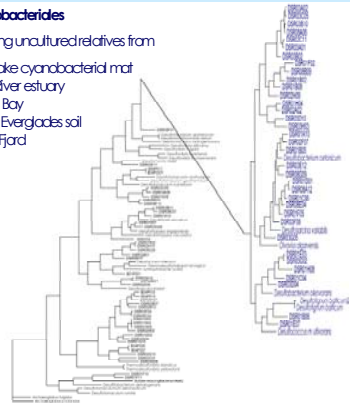
(Aarhus Bay and Kysing Fjord, Denmark; Seine River Estuary; Florida Everglades soil; Guaymas Basin hydrothermal vent). Trees constructed only from one or the other of the subunits allowed comparison with environmental datasets from other sites.



Based on *dsrAB* sequence analysis, the *Spartina* rhizosphere harbors a diverse community of sulfate-reducing bacteria, including.....

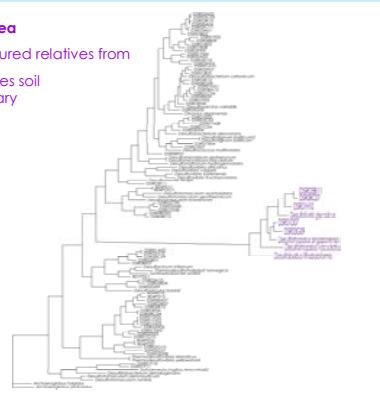
Desulfobacterides

including uncultured relatives from
Solar Lake cyanobacterial mat
Seine River estuary
Aarhus Bay
Florida Everglades soil
Kysing Fjord



Desulfobulbaceae

including uncultured relatives from
Florida Everglades soil
Seine River estuary

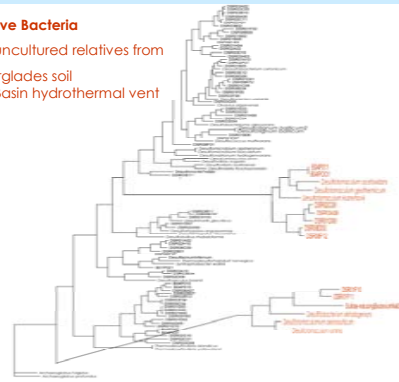


Methods

- Triplicate cores taken monthly March-October
- Extract DNA from top 4 cm with bead-beating, phenol-chloroform and ethanol precipitation
- Amplify with *dsr* specific primers (Wagner et al. 1998)
- Construct 3 clone libraries:
DNA from all months, Mar.-Oct. (clones DSR01, DSR02)
March only (clones DSR03)
August only (clones DSR08)
- Design internal primers and sequence 1.9kb full-length gene from ~400 clones on ABI 3730
- Alignment of nucleotides (ARB) and amino acids (MacClade)
- Construction of neighbor-joining trees from full-length *dsrAB* genes, subunit *dsrA* and subunit *dsrB* (Paup 4)

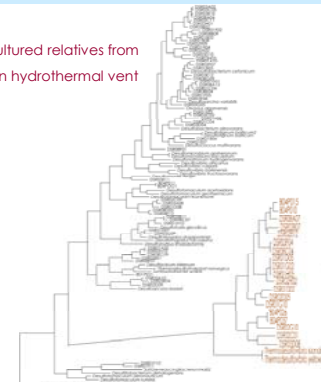
Gram Positive Bacteria

including uncultured relatives from
Florida Everglades soil
Guaymas Basin hydrothermal vent



Nitrospirales

including uncultured relatives from
Guaymas Basin hydrothermal vent



Summary

- The functional *dsr* gene is a specific indicator of sulfate-reducing prokaryotes.
- A *dsr* database has been constructed which includes SRP from a variety of environments.
- *dsr* genes do not distinguish between SRP present prior vs. those present late in the *Spartina* growing season.
- Full-length sequence data can now be used for probe/primer development to detect gene expression associated with sulfate-reducing activity.

References

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