

Analysis of Bacterial Communities in Seagrass Bed Sediments by Double-Gradient Denaturing Gradient Gel Electrophoresis of PCR-Amplified 16S rRNA Genes

J.B. James¹, T.D. Sherman² and R. Devereux¹

(1) Office of Research and Development, US Environmental Protection Agency, NHEERL-Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA

(2) Department of Biology, University of South Alabama, Mobile, AL 36688, USA

Received: 30 January 2006 / Accepted: 8 April 2006 / Online publication: 10 June 2006

Bacterial communities associated with seagrass bed sediments are not well studied. The work presented here investigated several factors and their impact on bacterial community diversity, including the presence or absence of vegetation, depth into ~~asson.ogy~~ Double

seagrass decline and enhance efforts in seagrass restoration. It is therefore important to define the dynamics of bacterial communities associated with seagrasses.

Many strategies for examining microbial diversity entail analysis of ribosomal RNA (rRNA) sequences, including denaturing gradient gel electrophoresis (DGGE) [26, 29]. DGGE is a genetic fingerprinting technique that enables the separation of equally sized DNA fragments

Table 2. Assignment of banding patterns to month groups by jackknife analysis (Dice coefficient)

Month collected	Banding patterns (%) assigned to groups		
	Feb	Jun	Oct
Feb	81.4	4.7	14.0
June	24.4	57.8	17.8
Oct	21.2	6.1	72.7

ERCCs of patterns to host class are in boldface. The mean ERCC was 70.7%.

ison. Within Bionumerics, optimization refers an adjustment of bands beyond normalization and was necessary when imperfect normalization resulted in residual shifts. Likewise, tolerance refers to the total distance that bands in different lanes differed by before they were determined to be distinct. The default values were used for optimization and tolerance and were 0.17 and 3.5%, respectively. At these values, bands that differed by more than 3.5% of

use31 7 (isea)2 -14on:use31TJ34pF.0468d3c (necreBD 7151p (3in eBD 7151ds, 222T64.7 (the22-113.s1dimeBD 7151n7the22 (Ge6ba

sediment. Although this study does not refute the fact that depth into sediment greatly influences the microbial community, it highlights the fact that such influences may not be consistent enough over the seasons or in the presence or absence of vegetation as to be elucidated using this community DNA fingerprinting technique. It is also possible that the DGGE method is insufficiently sensitive to detect differences with depth.

The ERCC for assignments based on month (as a proxy for season) was high, and a likely explanation is that the bacterial communities for a given month were likely responding to the seasonal status of the seagrasses. Microbial activities in seagrass bed sediments show strong seasonality and are highest when the plants are actively growing [10, 38].

The ERCC for assignments based on the presence of vegetation was also significantly higher than random. Seagrass bed sediments support higher numbers of bacteria and greater bacterial activities than nonvegetated sediments because of enrichment with organic carbon [10, 19, 20, 38]. Bacterial communities in unvegetated sediment do not experience these inputs and would be expected to differ at some level from the seagrass bed sediment communities. In contrast, Bagwell et al. [2] obtained one reproducible DGGE banding pattern for *nifH* sequences amplified from seagrass bed sediments in an oligotrophic environment and nearby nonvegetated sediments. Similarly, Smith et al. [38] concluded that the community composition of sulfate-reducing bacteria, based on comparisons of dissimilatory sulfite reductase genes, at the same site used in the present study did not vary substantially between vegetated and unvegetated sediments. Smith et al. [38] did find some small groups of sulfate-reducing bacteria unique to either the vegetated or nonvegetated sediment and suggested they could represent responses to available carbon sources, either root exudates or benthic algae, respectively. Similarities in microbial communities between vegetated and non-vegetated sediment are therefore to be

2. Bagwell, CE, La Rocque, JR, Smith, GW, Polson, SW, Friez, MJ, Longshore, JW, Lovell, CR (2002) Molecular diversity of diazotrophs in oligotrophic seagrass bed communities. *FEMS Microbiol Ecol* 39: 113–119
3. Borum, J, Pedersen, O, Grave, TM, Frankovich, TA, Zieman, JC, Fourqurean, JW, Madden, CJ (2005) The potential role of plant oxygen and sulfide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *J Ecol* 93: 148–158
4. Bulthuis, DA (1994) Light environments/implications for management. In: Wyllie-Echeverria, S, Olson, AM, Hershman, MJ (Eds.) EPA 910/r-94-004. Seagrass Science and Policy in the Pacific Northwest: Proceedings of a Seminar Series, pp 23–27
5. Caffrey, JM, Kemp, WM (1991) Seasonal and spatial patterns of oxygen production, respiration and root-rhizome release in *Potamogeton perfoliatus* L. and *Zostera marina* L. *Aquat Bot* 40: 109–128
6. Campbell, R, Greaves, MP (1990) Anatomy and community structure of the rhizosphere. In: Lynch, JM (Ed.) *The Rhizosphere*. John Wiley & Sons, Chichester, England, pp 11–34
7. Carlson, PR, Yarbro, LA, Peterson, BJ, Ketron, A, Arnold, H,