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

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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 sediment, and season. Double-gradient denaturing gradient gel electrophoresis (DG-DGGE) was used to generate banding patterns from the amplification products of 16S rRNA genes in 1-cm sediment depth fractions. Bioinformatics software and other statistical analyses were used to generate similarity scores between sections. Jackknife analyses of these similarity coefficients were used to group banding patterns by depth into sediment, presence or absence of vegetation, and by season. The effects of season and vegetation were strong and consistent, leading to correct grouping of banding patterns. The effects of depth were not consistent enough to correctly group banding patterns using this technique. While it is not argued that bacterial communities in sediment are not influenced by depth in sediment, this study suggests that the differences are too fine and inconsistent to be resolved using 1-cm depth fractions and DG-DGGE. The effects of vegetation and season on bacterial communities in sediment were more consistent than the effects of depth in sediment, suggesting they exert stronger controls on microbial community structure.

Seagrasses are submerged flowering plants that grow rooted in coastal sediments of intertidal, subtidal, or shallow coastal zones [12], including the waters contiguous with the Gulf of Mexico in the USA. Seagrasses found in the northern Gulf of Mexico, and in Santa Rosa Sound in northwest Florida in particular, include *Halodule wrightii* (shoal grass) and *Thalassia testudinum* (turtle grass), among others. Seagrass habitats are in decline, both in the USA and around the world [1, 30]. Seagrass bed coverage in Santa Rosa Sound has declined from historical levels, and their preservation has been the subject of study and monitoring, with the hopes of restoring lost habitats [22, 23, 34]. Seagrasses provide several vital estuarine ecosystem functions, and their protection and restoration are important. Seagrasses link

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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)

	Banding patterns (%) assigned to groups		
Month collected	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

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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 *nif*H 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 nonvegetated sediment are therefore to be pat.374 0 Td(10)TjEM-194.9 (could)]TJ0 -1.138 TDarbe.5 s]TJ/T1_2 1iTJc196.8 (se)(a

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