

Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD)

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Abstract

Similarity among a number of aquatic microbial communities was examined using randomly amplified polymorphic DNA (RAPD), a common polymerase chain reaction (PCR)-based DNA fingerprinting technique. After amplification of whole-community DNA extracts, the PCR products were resolved by agarose gel electrophoresis and the band patterns compared to determine percent similarity. Twelve different primers were used to amplify approximately 100 fragments (total) from each DNA sample; the bands were scored as present or absent and the similarity between each sample was determined using Jaccard's coefficient. From this information, dendrograms were constructed and a bootstrapping procedure was used to assess how well supported the tree topologies were. Principal component analyses were also conducted as a means of visualizing the relationships among samples. Results obtained for two different experimental systems (a pair of tidal creeks and several wells in a shallow groundwater aquifer) correlated well with the temporal and spatial variations in environmental regime at the sites confirming that arbitrarily primed PCR-based DNA fingerprinting techniques such as RAPD are useful means of discriminating among microbial communities and estimating community relatedness. Moreover, this approach has several advantages over other DNA-based procedures for whole-community analysis; it is less laborious and uses smaller quantities of DNA, making it amenable to sample-intensive monitoring, and it does not depend on culturing or the use of selective PCR primers. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The abundance and diversity of microorganisms in a given environment is typically enormous. As a

result, it is not possible to get a complete sense of the relative numbers and identities of the constituent organisms in a microbial community with any of the currently available analytical techniques. It is similarly impossible to thoroughly understand the function of each individual organism type, the specific interactions that may exist between populations, or the independent influences organisms may have on ecosystem processes. This inability to completely categorize the constituents of a community has hampered the efforts of microbial ecologists to

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investigate fundamental ecological concepts such as community diversity, succession, redundancy, or stability.

Culture-based studies provide the framework from which microbial ecologists derive much of their current understanding of microbial interactions and community dynamics; however, the fraction of organisms that have been cultured is thought to be less than 1% of the total (Holben, 1997). In order to comprehend the full extent of the relationships within a microbial community, and between a community and its surroundings, researchers must be able to evaluate attributes for an entire community without relying upon microbial growth for detection. This need has led to the development of several approaches that use whole-community samples for analysis with much of the research focusing on the use of whole-community DNA extracts (Holben, 1997; Griffiths et al., 1996; Ogram and Feng, 1997). Commonly used techniques include DNA hybridization (Griffiths et al., 1996; Lee and Fuhrman, 1990; Lee and Fuhrman, 1991), percent G + C content (Holben and Harris, 1995), DNA reassociation (Torsvik et al., 1994; Britten et al., 1974), and RFLP (restriction fragment length polymorphism) (Stahl, 1997). The primary limitation of these procedures is that they require rather large amounts of relatively pure DNA for analysis. The need for large quantities of DNA often means that sample collection can be very time consuming (e.g. filtering large volumes of water), and that samples may need to be gathered over a relatively large area (e.g. several grams of soil or sediment), making it impossible to examine small-scale spatial differences in community structure. Moreover, the analyses themselves are very time consuming further limiting the feasibility of large and comprehensive studies of microbial communities.

The application of polymerase chain reaction (PCR) to microbial ecology has helped to eliminate some of these problems, in particular those resulting from the need for large quantities of DNA, and has eliminated the need for extensive concentration of cells from environmental samples. PCR has proven especially useful in the study and comparison of the DNA sequences of the genes coding for rRNA. Some of the rDNA studies compare individual sequences to a database of previously encountered

(generally cultured) organisms (Devereux and Willis, 1995) in order to monitor the distribution of “phylotypes” (distinct groups of related DNA sequences) in the environment (Wise et al., 1997); inevitably, this comparison, and the database construction, includes some cultural bias. Other researchers studying rDNA have taken a different approach—without attempting to link the sequences with known taxa, they examine the diversity of “fingerprints” derived from manipulation of rDNA sequences (e.g. ARDRA—Amplified Ribosomal DNA Restriction Analysis (Weidner et al., 1996; Massol-Deya et al., 1995), DGGE—Denaturing Gradient Gel Electrophoresis (Ferris et al., 1996; Øvreås et al., 1997), and ‘T’-RFLP—Transfer RFLP (Liu et al., 1997)). However, these techniques have still been criticized because the “universal PCR-primers” used for the PCR amplification of the 16S or 23S rRNA genes may not amplify fragments from all community members with equal efficiency (Pepper and Pillai, 1994). Nevertheless, such approaches provide a significant improvement over the 0.1–1% of the community accessible with standard, culture-based techniques.

The present research examined another PCR-based approach, DNA fingerprinting, as a means of comparing microbial communities, and focused on the development of a community-level technique that uses very small quantities of DNA and has no need for culturing or the use of selective PCR primers such as those used in rDNA studies. In particular, RAPD (randomly amplified polymorphic DNA) (Williams et al., 1990) was used to compare a number of aquatic microbial communities and quantify their overall similarity. RAPD employs short primers of arbitrary sequences to amplify random portions of the sample DNA by PCR. Since each primer is short, it will anneal to many sites throughout the target DNA; a fragment is amplified whenever two of these primers anneal close enough together and in the proper orientation with respect to one another. Individuals that have different sequences will have primers that anneal in different places and therefore produce a different spectrum of fragments from the PCR, a different genetic “fingerprint”. Because each primer generates relatively few (5 to 15) distinct bands when separated on an agarose gel, several reactions must be run, using

several different primers, and the results combined to obtain the desired number of markers. Pooled results can then be compared between samples and percent similarity computed. Using multiple primers also helps ensure that a sufficiently large region of the target DNA is scanned when an estimate of overall variance between samples is desired (Ogram and Feng, 1997). Typically, 10 to 15 primers (~100 bands) are required for statistical comparison of samples using RAPD markers (Xia et al., 1995; Demeke and Adams, 1994).

RAPD is commonly used to differentiate among closely related strains of bacteria (Busse et al., 1996), and use of this technique for mixed genome samples has been suggested, though experimental applications are rare (Xia et al., 1995; Bruce et al., 1992; Picard et al., 1992; Moll et al., 1998). Previous work with individual genome samples has shown that RAPD provides results that are very consistent with other molecular genetic techniques including RFLP (Paffetti et al., 1996; Karp et al., 1996), AFLP—amplified fragment length polymorphism (Powell et al., 1996), and DNA hybridization (Tanaka et al., 1994).

In this research, the usefulness of RAPD for comparing microbial communities was evaluated in two aquatic systems. The first was a pair of tidal creeks (Hungars Creek and Phillips Creek) that were compared twice during the summer of 1997 (June and July). The second application examined the groundwater microbial communities from anaerobic and aerobic zones of a shallow coastal plain aquifer on Virginia's eastern shore.

2. Materials and methods

2.1. Site descriptions

2.1.1. Tidal creeks

Creek water samples were collected from the Virginia Coast Reserve Long Term Ecological Research site on Virginia's eastern shore. Two marsh creeks, one on each side of the Delmarva peninsula, were sampled during the summer of 1997 (June and July). Phillips Creek, on the seaside of the peninsula, contains an extensive marsh system (135 ha) while Hungars Creek, on the Chesapeake bay side, is much

wider and shallower with a smaller area of marsh (35 ha). The two creeks are separated by less than 7 km and, consequently, may be compared without major differences in regional or local climate, land-use patterns, or tidal amplitude (MacMillin et al., 1992). A number of biological and physical–chemical parameters differ between these two creek systems including higher organic matter, bacterial biomass, abundance, and productivity in Hungars Creek and greater nutrient concentrations (e.g. phosphate, ammonia, nitrate, and nitrite) in Phillips Creek (MacMillin et al., 1992). The dissolved organic carbon (DOC) source also differs for the two systems; Hungars Creek's DOC is primarily derived from phytoplankton while marsh grass/detritus is the main DOC source for Phillips Creek.

2.1.2. Groundwater

The groundwater samples for this study were collected from a shallow coastal plain aquifer near Oyster, VA, USA. The research site occupies an abandoned agricultural field (1.7 ha) that overlies distinct regions of anaerobic and aerobic groundwater. Samples were collected in August 1997 from four wells, two aerobic (B3 and C3) and two anaerobic (D1 and W2). The groundwater chemistry between these two regions of the field differs substantially, primarily in the amount of dissolved oxygen, alkalinity, nitrate, ammonia, and dissolved iron present (Knapp, 1997). Average microbial abundance, determined by acridine orange direct counts, also differs among the wells (D1: $5 \cdot 10^6$ cells/ml; W2: $3 \cdot 10^7$; C3 and B3: $3 \cdot 10^5$) (Lancaster and Mills, 1995).

2.2. Sample collection

To isolate the microbial community for analysis, water samples were filtered onto 0.22 μm pore-size polycarbonate membranes after prefiltration through AE glass fiber filters which removed particulate matter and eukaryotic organisms. Five and 20 l of water were filtered for the groundwater and creek samples respectively. The DNA from the creeks was collected in conjunction with another research project so a larger volume of water was sampled at that site. At both sites, the amount of DNA collected was far in excess of that necessary for RAPD community

profiling (see below). For the groundwater sites, three replicate 5-l fractions were collected in the field so within-well and between-well variance could be compared. All filters were transported to the laboratory on dry ice and stored at -70°C until the DNA was extracted.

2.3. Extraction of DNA

2.3.1. Recovery of cells from filters

DNA from the creek sites was extracted directly from the filters (Fuhrman et al., 1988), quantified using UV spectroscopy, and stored at -20°C . For extraction from the groundwater samples, bacteria were recovered from the filters by vortex mixing and repeated centrifugation prior to extraction. Specifically, the membranes were cut with scissors into small pieces using aseptic technique, resuspended in ~ 30 ml of sterile water, and vortex-mixed for 2 min. The supernatant was collected and the filter pieces were resuspended and mixed a second time. The two liquid fractions were pooled and filtered through an AE glass fiber filter to remove small pieces of the membrane. The bacterial suspension was then centrifuged in two 50-ml centrifuge tubes at 4°C at 30 000 g for 30 min. All but 5 ml of the supernatant was discarded; the tube contents were resuspended and the liquid from the two tubes combined. This consolidation procedure was repeated several times as necessary until the cell suspension could be transferred into a single 1.5-ml microcentrifuge tube. Acridine orange direct counts (Hobbie et al., 1977) were then performed on this suspension in order to normalize the number of cells that entered the DNA extraction procedure, providing a means of standardizing the amount of DNA entering each PCR reaction.

2.3.2. DNA extraction from groundwater cell suspensions

Depending on the concentration of cells in a given suspension, different volumes were removed corresponding to a total of approximately $5 \cdot 10^7$ cells. Cell pellets were collected from these different volumes by centrifuging in a microcentrifuge for 5 min at 13 200 rpm. After discarding the supernatant, 500 μl of buffer (0.02 M Tris (pH 7.8), 0.02 M EDTA (pH 8.0), 0.5% sodium dodecyl sulfate) was

added and the pellet resuspended by vortex-mixing. A boiling water bath was then used to lyse the cells (5 min) and 500 μl of phenol–chloroform–isoamyl alcohol (25:24:1) was added. The mixture was then centrifuged for 5 min as described above. The aqueous layer was transferred to a new tube and, after a second phenol extraction, the DNA was precipitated using an equal volume of cold isopropanol (500 μl). The DNA was centrifuged for 2 min and the pellet was washed twice with ice cold 70% ethanol. The DNA was dried, resuspended in 25 μl of sterile, filtered, deionized water, and stored at -20°C .

2.4. RAPD amplification

2.4.1. Amplification conditions

The PCR reactions were performed using the protocol from Williams et al. (1990) with slight modification. Specifically, 5 μl of the template DNA was added to a 20- μl reaction mixture containing: 10 mM Tris–Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl_2 , 100 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM primer, and 1 unit of *Taq* DNA polymerase (Perkin–Elmer). Amplifications were performed in a Perkin–Elmer 480 DNA Thermal Cycler programmed for 45 cycles of 1 min at 94°C , 1 min at 36°C , and 2 min at 72°C . Amplification products were separated by electrophoresis in 1% agarose gels, stained with ethidium bromide, and photographed under UV light.

2.4.2. DNA template concentration

For the tidal creek samples, several dilutions of the original DNA template were tested to determine the appropriate DNA concentration for the PCR reaction. Serial dilutions of the original stock were prepared in sterile, filtered, deionized water, and 10^{-3} , 10^{-4} , and 10^{-5} dilutions all generated similar profiles; this trend was confirmed for five different primers. A 10^{-4} dilution, which corresponded to 225 pg of DNA per PCR, was used in all subsequent amplifications of the creek community DNA. For each creek DNA sample, three replicate PCR reactions were performed in order to assess the repeatability of the RAPD procedure.

For the groundwater samples, 10^{-2} , 10^{-3} , and 10^{-4} serial dilutions of the original DNA stock all

produced similar RAPD fingerprints (screened using five primers), and a 10^{-3} dilution was used in all further reactions. This dilution corresponded to amplification of DNA extracted from 2000 cells, an original sample volume from the well of 0.24 μl (using the average bacterial concentration of the four wells = $8.9 \cdot 10^6$ cells/ml). Additionally, since the 10^{-4} dilution produced similar profiles, it is possible that as few as 200 cells (equivalent to an original sample volume of 0.02 μl) could have been used in the RAPD reaction.

2.4.3. Primer selection

Forty different primers (purchased from Operon Technologies) were tested for their ability to provide readily interpretable and reproducible RAPD profiles. Fourteen of these were selected for use in this study (Table 1). Though the choice of primers is somewhat subjective, criteria such as number of bands produced, clarity and distinctness of bands, and reproducibility of the RAPD fingerprints were used in the selection process. Another criterion that should be considered when selecting a primer is its ability to detect differences among the samples of interest; a primer that gives identical band patterns for all samples does not help discriminate among communities. This final criterion did not significantly influence primer selection in the present study as 39

of the 40 primers screened showed significant differences when tested on groundwater samples from a pair of wells, W2 (anaerobic) and C3 (aerobic).

From the 40 primers screened, 11 were chosen for use with the groundwater samples. With these, 80 different amplification products (fragments) were observed of which 91% were polymorphic. Individual well samples contained between 29 and 49 of these bands.

For the creek samples, the same 11 primers were tested and nine of them were successfully applied. Three additional primers, not tested on the groundwater communities, were chosen for use with creek samples. These 12 primers produced a total of 101 distinguishable bands and an individual sample contained between 44 and 53 of these bands. Of these 101 bands, 92% were polymorphic.

2.5. Analysis of DNA fingerprints

Each fragment visualized on the agarose gels was treated as a unit character, scored as present or absent in each sample, and used to construct a distance matrix and dendrogram based on the Jaccard coefficient, which calculates the proportion of positive bands shared by each sample pair (Sneath and Sokal, 1973). In order to assess the statistical significance of the groupings and subgroupings in each dendrogram, a bootstrapping procedure was employed (Stackebrandt and Rainey, 1995; Swofford et al., 1996). This technique randomly resamples the data, alternately truncating or rearranging the original data set, and computes the fraction of times that a branching point appears in the recomputed trees. This is repeated a number of times (usually between 100 and 1000) and the larger the reported bootstrap value, the proportion of times a particular node appeared in the recomputation process, the greater the significance of the branching point.

In this research, the bootstrapping was accomplished by first using “SeqBoot” in PHYLIP to bootstrap the presence/absence data sets 100 times (Felsenstein, 1993). Each of the resultant data sets was then fed into the clustering program of SPSS (Version 7.5.1) and similarity matrixes were determined using Jaccard’s coefficient. Next, distance matrixes (distance = 1 minus similarity) were computed and the “Neighbor” program of PHYLIP was

Table 1
Sequences and average number of bands generated for RAPD profiling of creek and groundwater samples

Operon I.D.	Sequence	Creeks (No. bands)	Groundwater (No. bands)
C 4	CCGCATCTAC	6	5
D 5	TGAGCGGACA	7	13
F 4	GGTGATCAGG	13	14
F 7	CCGATATCCC	7	N.C. ^a
F 5	CCGAATTCCC	6	N.C.
F 3	CCTGATCACC	9	4
F 1	ACGGATCCTG	5	7
F 14	TGCTGCAGGT	6	N.C.
S 10	ACCGTTCCAG	10	5
S 13	GTCGTTCTCG	13	6
S 14	AAAGGGGTCC	N.C.	7
T 7	GGCAGGCTGT	7	5
X 5	CCTTCCCTC	12	5
X 16	CTCTGTTCGG	N.C.	9

^a “N.C.” indicates that data were not collected using a given primer.

used generate a 100 different recomputed trees using UPGMA clustering (Norusis, 1994). The “bootstrap value”, the proportion of recomputed trees that contain a given node, was then determined by feeding the tree file from “Neighbor” into the “Consense” subroutine of PHYLIP. In addition to cluster analysis, principal component analysis (SPSS, Version 7.5.1) of the original data was performed and diagrams of the first two principal components were constructed. Though principal component analysis is not necessarily suited for use with binary data such as these, it is often used as a supplemental means of visualizing the relationships from RAPD profiles (Demeke and Adams, 1994).

3. Results and discussion

The results of the present study suggest that RAPD is a useful technique for studying variation among microbial communities. For the creek water samples, RAPD showed distinct differences between the two sites and the two times (Figs. 1 and 2). For

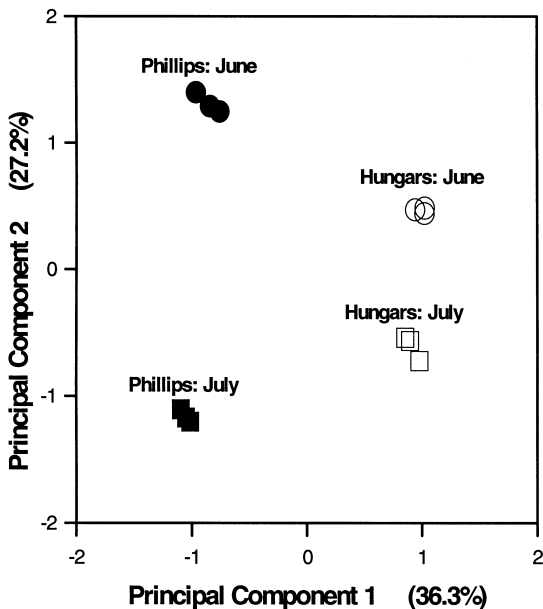


Fig. 1. Principal component diagram of the creek samples. The three points for each site represent replicate PCR reactions of the same DNA template. The percent of variance explained by each principal component is also listed.

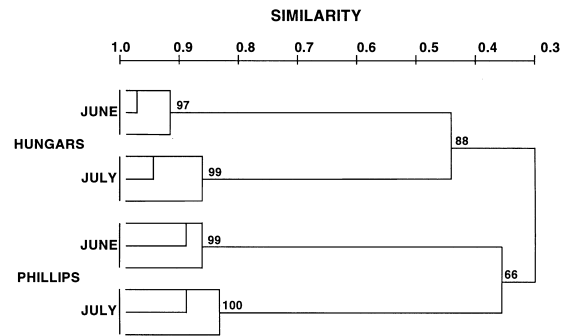


Fig. 2. Dendrogram displaying the results of a cluster analysis using Jaccard’s coefficient as a measure of similarity among the creek samples. The scale along the top represents percent similarity; the three prongs for each site represent replicate PCR reactions of the same DNA template. The numbers at each node are the bootstrap values (bootstrap performed using 100 replications).

the groundwater samples, differences between the anaerobic and aerobic zones of the aquifer could be distinguished (Figs. 3 and 4). In both investigations, the RAPD results correlated well with differences in the physical–chemical properties of the various sampling sites.

3.1. Tidal creeks

Hungars Creek and Phillips Creek were compared twice during the summer of 1997 (June and July), and Figs. 1 and 2 show the results of the principal

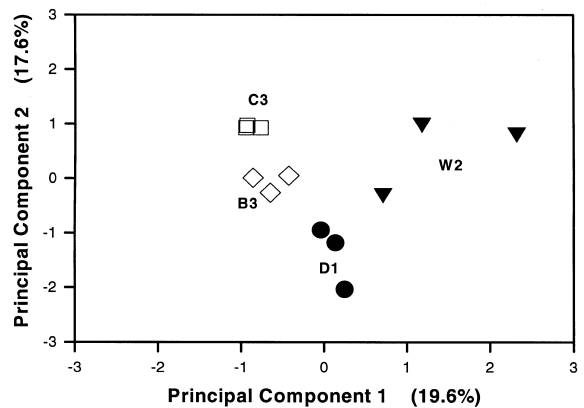


Fig. 3. Principal component diagram of the groundwater samples. The three points for each site represent independent replicas (separate 5-l fractions of water collected from the well). The percent of variance explained by each principal component is also listed.

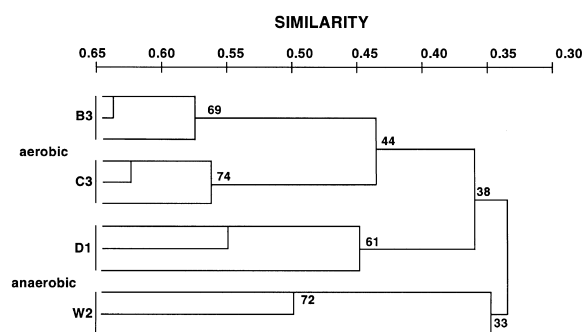


Fig. 4. Dendrogram displaying the results of a cluster analysis using Jaccard's coefficient as a measure of similarity among the groundwater samples. The scale along the top represents percent similarity and the three prongs for each site represent independent replicas (separate 5-l fractions of water collected from each well). The numbers at each node are the bootstrap values (bootstrap performed using 100 replications).

components and cluster analysis, respectively. In general, temporal changes in community structure were less for Hungars Creek than for Phillips (Hungars Creek—46% similar for June/July, Phillips Creek—38%); overall, the two creeks were 30% similar to each other.

The repeatability of the RAPD procedure was examined by profiling each creek sample three times (three PCR reactions using the same DNA template). PCR repeatability was quite high with replicate community profiles averaging 89% similar (101 bands compared). Additionally, replicate reactions always clustered together in the dendrogram (Fig. 2) and grouped tightly on the principal component plot (Fig. 1). One of the major problems reported by users of RAPD is artifactual variation in banding patterns (Ellsworth et al., 1993; Meunier and Grimont, 1993), though the magnitude of this problem varies greatly between laboratories (Penner et al., 1993). Nevertheless, the results presented here suggest that, with careful standardization of reagents and amplification conditions, the impact of variation among individual PCR reactions on overall community profiles is negligible.

The tree structure presented in Fig. 2 was analyzed using the bootstrapping procedure described above and all nodes were very well supported; each was found in >65% of the recomputed trees. Bootstrapping not only provided a measure of the support for each cluster, it also confirmed that the RAPD

procedure, as applied, generated enough bands to adequately describe the relationships among the samples; random subsampling of the data set did not significantly alter the observed pattern.

Based upon previous work comparing the bacterial dynamics in these tidal creeks (MacMillin et al., 1992; Lowit et al., 1998), it is not surprising to find that the microbial communities in Hungars and Phillips creeks differ structurally. Though separated by only a few kilometers, their differences in nutrient concentration, organic matter content, and DOC source could support different microbial inhabitants; the two creeks were only 37% similar in June and 30% similar in July. MacMillin et al.'s (1992) work also noticed significant differences in bacteria abundance between June and July, and differences in environmental regime between the two months (i.e. elevated nutrient availability and higher water temperatures in July) suggest that community structure may differ temporally. It is especially interesting to note how the different samples were separated by the two major principal components (Fig. 1); principal component 1 consisted of bands that separated the creek communities by location, and principal component 2 consisted of bands that separated the creek communities by time.

3.2. Groundwater samples

Similar statistical analyses were performed to analyze the RAPD profiles for the groundwater communities. Aerobic wells (C3 and B3) were more similar to one another than to either of the anaerobic sites (W2 and D1) (Figs. 3 and 4). Though the variability among replicate well samples was larger than for the creek samples, the dendrogram (Fig. 4) still showed a clear separation consistent with the observed patterns in groundwater chemistry at the site (Lancaster and Mills, 1995; Knapp, 1997). Historically, well W2 displays a very unique pattern in groundwater chemistry, compared to the other anaerobic wells (e.g. higher DOM, lower dissolved iron, and methane emission [Mills, unpublished data]), and it was also the most unique well using RAPD profiling. Similarly, the aerobic wells have nearly identical water chemistry, are physically separated by only 10 meters, and clustered tightly as anticipated.

A bootstrapping procedure was again used to determine how well supported the tree topologies were (Fig. 4); these bootstrap values were generally lower than for the creek water samples, indicating that the overall differences displayed in the dendrogram of the wells were not as significant as those for the tidal creeks. Confidence in this tree structure may be improved by increasing the number of RAPD bands compared; for the groundwater samples only 80 were used (as opposed to 101 for the creek samples) and greater separation and more stable clusters might have been obtained by using a larger number of bands. Other studies have also shown that at least 100 bands are necessary when making RAPD comparisons such as this (Demeke and Adams, 1994).

The three replicates compared for each well represent independent samplings (separate 5 l fractions of water) and show a much greater variability than was observed for the creek samples. It is important to recall, however, that the three values reported for each of the creek communities are replicate PCR reactions from a single DNA sample and do not describe any of the within-site variability present in these tidal creeks, nor do they reflect any of the variability from the DNA extraction procedure. The within-site variance for the groundwater samples was slightly higher than anticipated and the influence of small-scale spatial heterogeneity within the aquifer may partly explain this. Although an attempt was made to empty the wells of any stagnant water before sample collection, insufficient purging could mean that each fraction of water collected represented a slightly different community experiencing different environmental conditions. At each well, the most unique replicate was usually the first sample collected (3/4 times), suggesting that the order of collection, and hence proximity of the sample water to the well opening and air–water interface, was important. It is easy to imagine that different fractions of well water could support different microbial inhabitants in different subhabitats. The fact that the anaerobic wells show a greater within-site variance relative to the aerobic ones (Fig. 4) helps confirm this as it is anticipated that within-well differences in oxygen availability would be more influential under hypoxic conditions.

One of the most promising aspects of the RAPD

technique was the small quantity of DNA required and the ease of the DNA extraction procedure. The extraction procedure used with the groundwater samples was a simpler, rapid, alternative to the Fuhrman approach used in the creek water samples and its successful use in this study demonstrates that relatively crude DNA preparations may be used to profile communities using RAPD. Though more variability was observed for replicate PCR reactions of the same groundwater sample (results not shown) compared to the repeated PCRs of a single creek sample (Fig. 2), the contribution of this variability to the overall variance within the groundwater wells was observed to be quite small.

Considering the small amount of DNA used in the PCR reactions, it is possible that much smaller volumes of water could be collected for an analysis of a microbial community. Our calculations suggest that enough DNA could be obtained from as few as $3 \cdot 10^4$ cells (which corresponds to $\sim 3.5 \mu\text{l}$ [$0.24 \mu\text{l} \times 15$ primers]) from each of the groundwater wells in this study). In theory, even smaller samples are possible; PCR has been used to amplify DNA from a single cell (Davis et al., 1994). Needless to say, such extremely small samples will not capture the genomic diversity that exists in natural communities. The implementation of RAPD as a means of studying microbial community structure means that sample size, and the spatial scale over which variability can be studied, are no longer limited by technical considerations. Using more traditional techniques, the measurements made of microbial community structure typically represent broad scale characterizations and rarely consider the small scale on which the populations may actually be interacting. However, the uneven distribution of microorganisms in the environment suggests that community structure may vary intensely, at small spatial scales, depending upon heterogeneities in the environmental matrix and localized nutrient availability. The very small sample size required for RAPD profiling of communities offers a means of examining the spatial scale of the variance in community structure which has not previously been available. Ultimately, a better understanding of this natural variation will help microbial ecologists as they try to make inferences about how structure influences other community level characteristics.

4. Conclusions

The results of the present study confirm that RAPD fingerprinting is a very useful means of comparing microbial communities. Analysis of two separate aquatic systems provided results consistent with historical knowledge of the sites' different environmental regimes. The RAPD approach has many advantages over other DNA based, community-level analyses including the fact that it does not involve culturing, is very rapid, and is simple to perform. The primers used in RAPD are not selective for specific organisms, groups of organisms, or genes, and can therefore provide a better representation of the entire community than more traditional PCR-based approaches (e.g. 16S rRNA). Many primers kits are commercially available (e.g. Operon Technologies, Genosys Biotechnologies) making primer screening and selection very easy. As with other PCR-based techniques, RAPD uses very small quantities of DNA, decreasing sample collection time and permitting the analysis of fine-scale spatial patterns in microbial community structure. Larger scale changes may also be monitored if appropriate sample volumes are used. PCR's suitability to small, mixed, degraded, and impure samples make it especially useful in environmental applications (Bej et al., 1994), and this research suggests that relatively crude DNA extracts may be used, as was done with the groundwater samples, further increasing the speed with which a community analysis can be performed. Moreover, the ease of RAPD analysis means that an appropriate number of replicates may be run using this procedure; with most other techniques the practical limits of replication fall short of what is necessary for adequate statistical significance.

The primary disadvantage to RAPD analysis is the large number of primers and PCR reactions that must be performed in order to obtain enough bands for statistical comparison of the community profiles. In response to this, we are investigating the application of other PCR-based DNA fingerprinting techniques (e.g. AFLP; Zabeau and Vos, 1993) in the analysis of microbial communities. Additionally, it is not clear at this time how sensitive the RAPD technique is at detecting rare organisms within bacterial communities and this must be considered as one draws

conclusions from RAPD data. Some have estimated the lower limit of detection to be 1% of the total DNA pool (Xia et al., 1995), but it is likely that this ratio will vary somewhat depending upon the complexity of the community.

While RAPD fingerprinting is a useful technique for comparing communities without the limitation imposed by culture dependent procedures, it does not provide any direct information about the constituents of the community being examined. But, other community-level analyses (e.g. DNA hybridization) cannot provide such information either. Other approaches (e.g. 16S rDNA) can provide information about the presence or absence of some strains but no technique exists to completely characterize the structure of bacterial communities. However, whole-community studies such as these are the only means of holistically examining microbial systems and evaluating how the entire suite of organisms changes with changing environmental parameters. In combination with more traditional approaches, DNA fingerprinting techniques may allow scientists to move beyond their inability to completely categorize the constituents of a community toward the development of a more complete understanding of the overall interactions among bacterial populations and between populations and the environment.

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