APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2003, p. 5192–5197 Vol. 69, No. 9
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.9.5192–5197.2003

Spatial Distribution of *Rhodopseudomonas palustris* Ecotypes on a Local Scale

S. J. Bent,[1] C. L. Gucker,[1] Y. Oda,[2]† and L. J. Forney[1]*

University of Idaho, Moscow, Idaho,[1] and University of Groningen, Haren, The Netherlands[2]

Received 23 January 2003/Accepted 4 June 2003

The number, spatial distribution, and significance of genetically distinguishable ecotypes of prokaryotes in the environment are poorly understood. Oda et al. (Y. Oda, B. Star, L. A. Huisman, J. C. Gottschal, and L. J. Forney, Appl. Environ. Microbiol. 69:xxx–xxx, 2003) have shown that *Rhodopseudomonas palustris* ecotypes were lognormally distributed along a 10-m transect and that multiple strains of the species could coexist in 0.5-g sediment samples. To extend these observations, we investigated the clonal diversity of *R. palustris* in 0.5-g samples taken from the corners and center of a 1-m square. A total of 35 or 36 clones were recovered by direct plating from each sample and were characterized by BOX A1R repetitive element-PCR genomic DNA fingerprinting. Isolates with fingerprint images that were ≥80% similar to each other were defined as the same genotype. Among the 178 isolates studied, 32 genotypes were identified, and each genotype contained between 1 and 40 isolates. These clusters were consistent with minor variations found in 16S rRNA gene sequences. The Shannon indices of the genotypic diversity within each location ranged from 1.08 (5 genotypes) to 2.18 (13 genotypes). Comparison of the rank abundance of genotypes found in pairs of locations showed that strains from three locations were similar to each other, with Morisita-Horn similarity coefficients ranging from 0.59 to 0.71. All comparisons involving the remaining two locations resulted in coefficients between 0 and 0.12. From these results we inferred that the patterns of ecotype diversity at the sampling site are patchy at a 1-m scale and postulated that factors such as mixing, competitive interactions, and microhabitat variability are likely to be responsible for the maintenance of the similarities between some locations and the differences between others.

Sediment and soil environments are home to a large number and wide variety of bacteria. The compositions of the microbial communities have been studied at various levels of taxonomic resolution, and there has been an emphasis on overall community structure (8, 24) and guild structure, such as that of denitrifying (23) and ammonia-oxidizing groups (18). Diversity at individual sites and the spatial patterns of differences between the constituent organisms are still poorly understood. Looking at the distribution of closely related strains can help us understand the variability in habitats that drives natural selection and the adaptive evolution of species. The community structure of closely related strains has been studied in the context of establishing global and regional patterns (14, 17, 20), degrees of endemicity within sites (2, 5), and the resolution of analytical techniques (10), as well as determining clonal variation along gradients in soil (12) and sediment (29). However, information from systematic sampling within individual sites is sparse.

The patterns of intraspecies diversity that have been characterized thus far have yielded different structures for different species. For example, *Escherichia coli* tends to cluster into a small number of clonal populations (15, 25), whereas 3-chlorobenzoate-degrading isolates from soil have been shown to exhibit a greater range of differences between clones and a higher degree of endemicity (4). These different distribution patterns may be attributable to the different life history conditions to which these organisms have been subjected (28). The organisms which are generally subjected to K-selective conditions, such as those which generally live in low-nutrient conditions commonly found in soil, are often the organisms that are determined to have a more evenly divergent population structure (7, 11), whereas the species that have the opportunity for fast, R-selected clonal growth and dispersal can be seen as having a more quantized, discretely clonal population structure (1, 3, 13, 14, 25).

Rhodopseudomonas palustris is a metabolically versatile gram-negative species belonging to the alpha subclass of the *Proteobacteria* that is commonly found in soil and sediment (6). Strains of *R. palustris* have been shown to be able to degrade a wide variety of carbon compounds, including benzoate and 3-chlorobenzoate, which makes them interesting and potentially useful (22) and has resulted in genome sequencing by the United States Department of Energy's Carbon Management Program (*Rhodopseudomonas palustris* Genome Project [http://www.jgi.doe.gov/JGI_microbial/html/rhodopseudomonas/]). Strains of *R. palustris* are, in our experience, distinguishable by repetitive element PCR (rep-PCR), and the BOX A1R primer provides the most complex band pattern and the best resolution of closely related isolates. It has also been shown previously that genetic distances between bacterial isolates follow a lognormal distribution relative to the physical distance between sample sites at a 10-m scale and that multiple genotypes of a single species can coexist at a single sampling location (16).

In this study we investigated whether the correlation be-

* Corresponding author. Mailing address: Department of Biological Sciences, University of Idaho, 282 Life Sciences Bldg., Moscow, ID 83844-3051. Phone: (208) 885-2583. Fax: (208) 885-7905. E-mail: lforney@uidaho.edu.

† Present address: Department of Microbiology, University of Iowa, Iowa City, IA 52242.

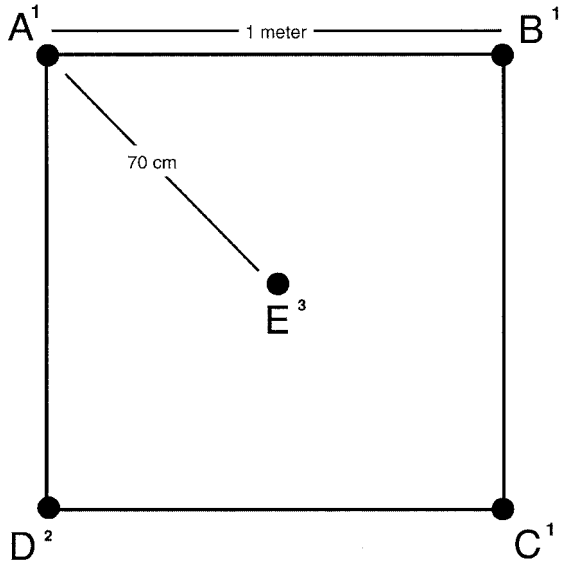

FIG. 1. Sampling scheme used in this study. The superscript numbers indicate which cluster of BOX A1R rep-PCR genotypes was predominant in each sample. Cluster 1 contained genotypes 1, 3, 4, 7, and 9; cluster 2 contained genotypes 2 and 6; and cluster 3 contained genotypes 5 and 8.

tween genetic distance and physical distance found by Oda et al. (15a) was supported at the 1-m scale. We analyzed the population structure of the purple nonsulfur bacterium *R. palustris* isolated by direct plating on selective media from the corners and center of a 1-m square using the 16S rRNA gene sequence to confirm species identity and rep-PCR to look at patterns of diversity within and between sampling sites.

Assessing the patterns of genetic variability found in this 1-m square could help us understand the distribution and diversification of closely related organisms competing for the same resources. This information can be used to determine the type of experiments required for future analyses of microbial populations in relatively undisturbed soil and sediment habitats.

MATERIALS AND METHODS

Isolation and culture conditions. Small (0.5-g) soil samples were taken from the corners and center of a 1-m square (Fig. 1) from wetland sediment (Haren, The Netherlands). From each location, 36 strains of *R. palustris* were isolated by direct plating on selective growth medium; a total of 180 strains were obtained. The sediment samples were resuspended in 10 ml of low-chloride minimal (LCM) medium (26), sonicated three times for 10 s each time, and then shaken at 150 rpm for 1.5 h. The sediment was allowed to settle for 30 min, and then three 10-fold dilutions were made and 0.1-ml portions of the initial suspension and each of the dilutions were spread on plates containing LCM medium supplemented with 2 mM benzoate and 0.1% sodium bicarbonate plus 1.5% agar. All incubations were performed anaerobically in the light at 30°C. After 2 weeks, 36 red to reddish brown colonies were picked for each site, and these colonies were grown in LCM medium liquid cultures containing 0.3% peptone and 0.3% yeast extract (LCMPY) plus 15 mM malate. A portion of each culture was frozen at −80°C with 20% (vol/vol) glycerol. All subsequent culturing and analysis were done with these stocks. We revived the stocks by scraping a small chunk of ice from the surface of each stock and streaking it on plates containing LCMPY plus 15 mM malate with 1.5% agar. After 1 to 2 weeks of incubation, a well-separated single colony was picked for each isolate and inoculated into 15 ml of LCMPY containing 15 mM malate in glass culture tubes with butyl stoppers that were evacuated and flushed with nitrogen.

Harvesting of cultures and DNA preparation. After 1 to 3 weeks of incubation, the cultures reached the maximum density. At harvest time, the culture density was rated semiquantitatively on a scale from 1 to 5 with 0.5-U intervals. We set aside 1 ml of each culture for a glycerol stock, and genomic DNA was extracted from the remainder of the culture by using a Wizard genomic DNA extraction kit (Promega, Madison, Wis.) and the accompanying standard gram-negative protocol. The DNA concentration was measured spectrophotometrically, and a working stock having a concentration of 50 ng/μl was prepared.

rep-PCR fingerprinting. rep-PCR was performed using three primer sets: BOX (A1R), enterobacterial repetitive intergenic consensus 1R, 2 (ERIC), and repetitive extragenic palindrome 1R, 2I (REP). The protocol of Rademaker et al. (19) was used with the Peltier cycler PCR cycle times. Each PCR product was prepared for loading by adding 0.2 volume of 6× loading dye (21). Gels were prepared by using 0.5× Tris-acetate-EDTA, 1.5% agarose, and 0.5 μl of ethidium bromide per ml. In order to compare methods, an additional complete set of BOX A1R rep-PCR (BOX-PCR) gels was prepared without ethidium bromide, and they were stained after they were electrophoresed in an ethidium bromide bath for 30 min; this was followed by 20 min of destaining under running water. The gels that were stained after electrophoresis more clearly showed the dimmer bands in the fingerprint patterns and were therefore used in all subsequent analyses. All of the gels were photographed with a gel documentation system digital camera (AlphaImager 1220) with UV transillumination, and the TIFF images were imported into BioNumerics 2.5 (Applied Maths, Kortrijk, Belgium) and normalized by using the 1Kb+ DNA ladder (Invitrogen, Carlsbad, Calif.). By using the spectral lane pattern between 300 and 3,000 bases, the isolates were clustered with a Pearson's coefficient similarity matrix, and the interrelationships were visualized by using an unweighted pair group using mathematical averages (UPGMA) tree. A cutoff of 80% similarity was used to define a genotype, and Shannon diversity indices ($-\Sigma p_i \ln p_i$, where p_i is the proportion of isolates in a given site that are of genotype i) and Morisita-Horn similarity coefficients $\{2 \times \Sigma(A_i \times B_i)/[N_A \times N_B \times (\Sigma A_i^2/N_A^2 + \Sigma B_i^2/N_B^2)]$, where A_i and B_i are the numbers of isolates of genotype i in samples A and B, respectively, and N_A and N_B are the total numbers of isolates in samples A and B, respectively (all Σ are summed from $i = 1$ to S, the total number of species)$\}$ were calculated based on these genotype classifications.

16S rRNA gene sequencing. Partial 16S genes were amplified from genomic DNA by using primers fD1 and rD1 (27) and the following PCR program: 95°C for 210 s and then 30 cycles of 94°C for 70 s, 56°C for 40 s, and 72°C for 130 s, followed by a 6 min extension at 72°C and a final soaking at 4°C. The PCR product was cleaned up with a QIAquick PCR cleanup kit (Qiagen, Valencia, Calif.). The sequencing reaction was performed by using BigDye Terminator 3 reaction reagents (ABI Biotech, Foster City, Calif.) and the protocol for PCR product with 10 μl of 1× reaction mixture, 20 ng of template, and the 907R primer (9). The sequencing reaction product was cleaned up by using Edge Biosystems gel filtration cartridges (Edge Biosystems, Gaithersburg, Md.), eluted with 20 μl of distilled H_2O, and precipitated and washed with isopropanol. The samples were reconstituted with formamide (ABI HiDi) and electrophoresed with an ABI Prism 3100 genetic analyzer. The sequences were aligned by using DNATools (S. W. Rasmussen, Copenhagen, Denmark), and any differences between sequences were confirmed by visual evaluation of the electropherogram trace file and resequencing if any differences were unclear.

Statistical analyses of phenotypic data. The cultures were assessed visually daily, and when they were determined to have reached the maximum density, we rated the density using a semiquantitative scale from 1 to 5 with 0.5-U intervals. The culture density values of the 133 isolates belonging to the nine major genotypes (the genotypes containing five or more isolates) were analyzed by using the Kruskal-Wallis test, standard analysis of variance (AOV) procedures, and Fisher's protected least significant difference (LSD) to determine significant differences between means; both major genotype and sampling site were used as populations. The formulae used were taken from Ott and Longnecker's *Statistical Methods and Data Analysis* (16a) and were entered into Excel (Microsoft, Redmond, Calif.) to build statistical calculators for the Kruskal-Wallis, AOV, and LSD procedures by using sum of squares calculations instead of Excel's built-in statistical routines.

RESULTS

Sediment samples from each of the corners (sites A to D) and from the center (site E) of a 1-m square were diluted and plated on selective medium. From each sample, 36 colonies of purple nonsulfur bacteria were identified based on colony pigmentation, picked, and grown in liquid culture. Our expectation based on previous sampling from this site (16) was that

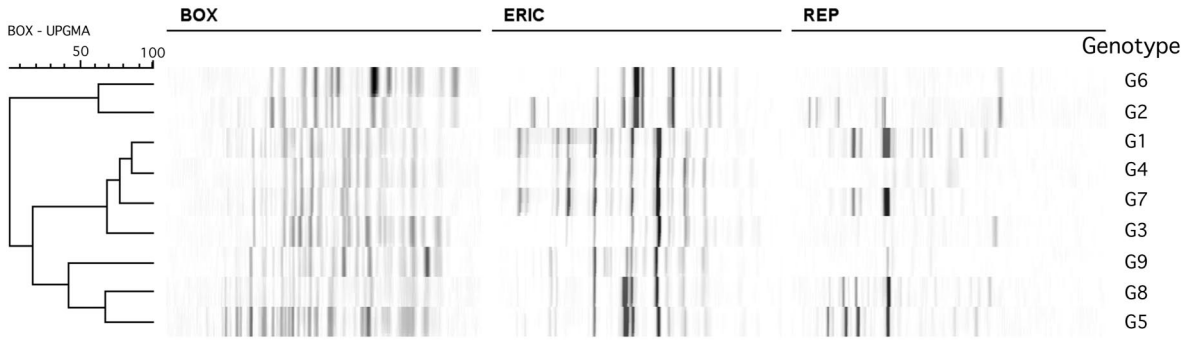

FIG. 2. ERIC, REP, and BOX-PCR fingerprints of a representative strain from each of the nine major genotypes found in this study. The genotypes are indicated by G followed by the number of the genotype. All dendrograms were produced by using Pearson's product-moment coefficient and a UPGMA clustering algorithm.

most of these isolates would be *R. palustris*. The patterns of DNA fragments in genomic fingerprints obtained by using the BOX A1R primer were used to group the isolates into genotypes. We chose the BOX A1R primer over the ERIC and REP primer sets because of the greater resolution of strains provided by the BOX-PCR band patterns (Fig. 2). The fingerprint patterns were analyzed by using a Pearson's product-moment correlation matrix and a UPGMA tree to cluster the isolates. An 80% similarity level was used to define the genotypes. We chose 80% because it was slightly lower than the outside boundary of variation seen with identical strains across different PCRs and gels (control data in Fig. 3). Of the 178 isolates obtained (two of the 180 isolates did not grow in liquid culture), 133 were assigned to nine major genotypes, each containing five or more isolates (Fig. 3). The rest of the groups contained four or fewer strains, representing minor genotypes and unique isolate genotypes.

We amplified and sequenced partial 16S rRNA gene sequences of each unique isolate and of at least 33% of the members of each genotype as defined by 80% similarity of the BOX-PCR fingerprints. All of the strains analyzed were shown to be phylogenetically closely related to *R. palustris* based on 600 bp of the 16S rRNA gene sequence corresponding (approximately) to *E. coli* 16S rRNA positions 250 to 850. Four phylotypes were found, all with at least 98.8% similarity and with at least 98% similarity to published sequences of type strain ATCC 17001 of *R. palustris*. All of the strains were therefore most likely *R. palustris* strains. The BOX-PCR clusters were congruent with the 16S rRNA gene sequence clusters; any group of strains whose fingerprints exhibited >40% similarity was subsumed within the group represented by a single 16S rRNA gene sequence (Fig. 4). There were differences between the two trees, but they were at deeper branches of the BOX-PCR similarity tree (<40%), where the topology was not strongly supported and therefore was neither informative nor problematic.

The diversity and distribution of all of the genotypes defined by the 80% similarity criterion were analyzed by using Shannon diversity indices and Morisita-Horn similarity coefficients. The Shannon diversity indices of the genotypes found at a sampling site ranged from 1.08 (only 5 genotypes present in 36 isolates) to 2.18 (13 genotypes present in 35 isolates); specifically, the Shannon indices for sites A to E were 1.71, 1.98, 1.29, 1.08, and 2.18, respectively. Morisita-Horn similarity coefficients, which were used to compare frequency distributions of genotypes between sampling sites, were calculated, and three sites showed clear similarity to each other, with values ranging from 0.59 to 0.71. All comparisons involving the other two sites exhibited values between 0.00 (no similarity) and 0.12 (Table 1). There were no intermediate values between 0.12 and 0.59, indicating that all pairs of sites sampled were either very different or very similar.

We used a semiquantitative assessment of maximum culture density in LCMPY as a phenotypic measure to serve as a proxy for physiological differences among the genotypes. For this analysis we used only the major genotypes (those containing five or more strains). The most striking difference noted even before statistical analysis was performed was the difference between the dense growth of cultures inoculated with strains from site D and the growth of cultures inoculated with strains from the other sampling sites. The density of cultures was strongly correlated with both the genotype of the isolates and the sampling site (site A, B, C, D, or E). AOV of the density scores showed that there was a highly significant ($P < 0.001$) difference between sampling sites. Fisher's protected LSD test with an alpha of 0.001 was used with both AOV results to determine significant differences between genotype and site means (Table 2). A Kruskal-Wallis test was also performed to ensure that the nonnormality of the density score distribution was not confounding the results, and this test also showed that the *P* value was less than 0.001, indicating that there were very significant differences between both sites and genotypes for mean culture densities. These tests indicated which mean culture density groups were statistically differentiable. When the cultures were grouped by sampling site, the cultures of strains from site D showed the highest mean density, followed by those from site E. Cultures of strains from sites A and B had the next lowest mean densities and were not statistically differentiable from each other, and the strains from site C had the lowest density. The analysis of density by site was also performed with the complete 178-isolate data set, which yielded virtually identical results (data not shown). The significant differences between the culture densities of isolates from different sites indicated that there were physiological differences

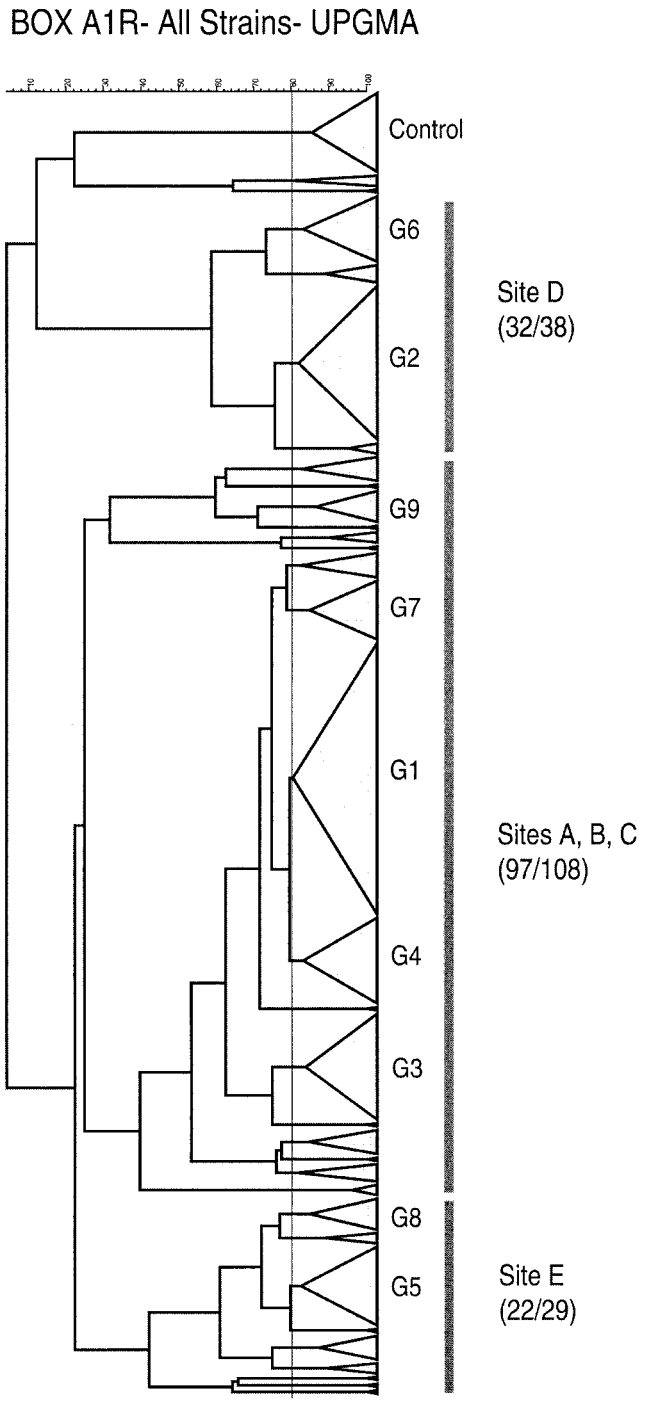

FIG. 3. Dendrogram of 178 BOX-PCR fingerprints of *R. palustris* strains produced by using Pearson's product-moment coefficient and a UPGMA clustering algorithm. The genotypes are indicated by G followed by the number of the genotype. Major clusters of strains associated with one site or group of sites are indicated by the gray vertical bars. The control data show the level (~85%) at which the fingerprint of a single strain (*Azorhizobium caulinodans* ORS-571) can vary across PCRs and gels. The vertical line indicates 80% similarity among fingerprints, which was used to define groups of related genotypes. Numbers in parentheses indicate the proportion of isolates in the indicated genotypes (G1 to G9) that came from the indicated site(s) (A to E).

between the isolates obtained from different sites and that these differences were correlated with genotypic differences. These results indicated that the strains isolated from the five sites were locally adapted to local differences.

DISCUSSION

In this study we used strains that were isolated from wetland sediment by selective plating for anaerobic growth on benzoate. All of the strains were shown to have 16S rRNA sequences similar to that of *R. palustris*, indicating the prevalence of *R. palustris* among the purple nonsulfur bacteria present at the site. Other taxa have been isolated previously by using the same medium and growth conditions, including *Rhodoferax fermentans* and *Rhodospirillum rubrum* (16). Consistent with our findings, the wetland area used for this study (Haren, The Netherlands) has previously had samples taken and selectively plated similarly, and a preponderance of *R. palustris* in the strains recovered was found (16).

The levels of diversity seen at each sampling site (between 5 and 13 distinct genotypes of *R. palustris* present in each 0.5-g sample of sediment) indicate that even within a single species, high levels of diversity can be maintained on a small spatial scale in heterogeneous environments such as soils and sediments. This diversity implies that there are many distinct microhabitats in which different strains or species of organisms can establish themselves without direct competition and thereby avoid the one niche-one organism competitive exclusion that would follow from such an interaction.

The genetic differences were not correlated with physical distance in a straightforward manner. The Morisita-Horn index values were either high (>0.59), indicating that there was strong similarity in the genotypes present and in the relative proportions between sites, or low (<0.12), indicating that there were strong differences. The differences were not related to distance, and instead we saw patchiness at this scale between our samples. Of the 21 genotypes observed that were represented by more than one isolate, 10 were composed entirely of isolates from sites A, B, and C, 7 were composed entirely of isolates from site E, and 1 was composed entirely of isolates from site D. There were three genotypes which contained isolates from more than one sampling sites. Genotype 2 contained 21 isolates from site D and 2 isolates from site E; genotype 9 contained 1 isolate from site A, 3 isolates from site B, and 1 isolate from site D; and genotype 12 contained 3 isolates from site D and 1 isolate from site E. The preponderance of strains endemic to sites or groups of sites suggests that either fitness differences under local conditions or physical isolation between sampling sites was responsible for the observed genotype distribution.

In our comparisons of the mean maximum culture densities of strains from the sampling sites and also of strains belonging to the major genotypes, we found highly significant phenotypic differences among the strains which we isolated. Comparison of the culture densities of strains from different sampling sites showed that there were metabolic differences between the numerically dominant strains found at each site. Given that the strains were almost exclusively endemic to one site (site D or E) or three sites (sites A, B, and C), the genotypes could be considered nested within sites for purposes of statistical anal-

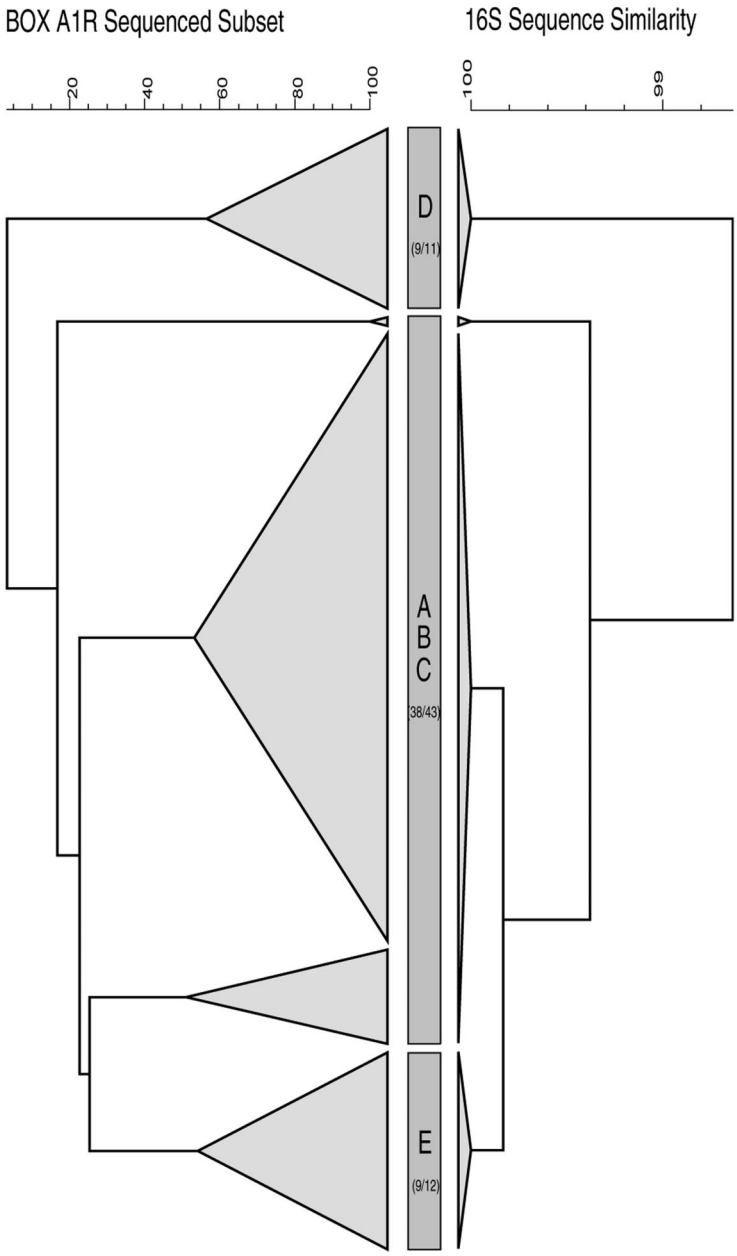

FIG. 4. Comparison of BOX-PCR and 16S rRNA gene sequences for a subset of strains (66 isolates). Both dendrograms were generated as described in the legend to Fig. 2. Numbers in parentheses indicate the proportion of strains from each similarity grouping that originated in the indicated site(s).

ysis. This nesting allowed us to interpret the significant phenotypic differences among the genotypes as due to correlations between site and genotype. We found that the genotypes associated with any one site had similar metabolic properties.

We cannot categorically attribute the phenotypic differences to habitat differences, as we did not perform physical and chemical analyses on the sediment samples; even if we had performed these analyses, other factors, such as nutrient flux, energy availability, and microhabitat variability (30), could have affected the actual conditions to which the organisms were subjected. The endemic distribution of differences be-

TABLE 1. Morisita-Horn similarity coefficients comparing the rank abundance of genotypes found at pairs of sampling sites

Site	Morisita-Horn coefficients with site:				
	A	B	C	D	E
A	1				
B	0.71	1			
C	0.70	0.59	1		
D	0.00	0.01	0.00	1	
E	0.00	0.00	0.00	0.12	1

TABLE 2. Mean maximum culture density for each sampling site (sites A to E) and mean separation groups (groups a to d) determined by using Fisher's LSD test with an alpha of 0.001

Site	Mean density[a]	LSD group
D	4.17	d
E	3.47	c
A	3.08	b
B	3.00	b
C	2.81	a

[a] Density values were obtained by visual assessment of the maximum culture density on a scale from 1 to 5 with 0.5-u increments.

tween physiological properties of strains from different sites suggests that there were selective forces involving metabolic characteristics of the bacteria driving the strain distribution. Many other biotic and abiotic factors must be involved in defining the competitive landscape, including the distribution of other organisms and physical and chemical properties of the local environment. The patterns which we observed in our data suggest that differences in outcomes of the competitive interactions in local environments lead to the distribution of genotypically and phenotypically distinct strains.

Further work needs to be performed to demonstrate the nature of the forces influencing strain distribution and to determine if similar patterns are found for different species and sampling sites. Genetic analysis performed in conjunction with physical and chemical analyses of the sediment samples may help elucidate some of the causes of the distributions of closely related bacterial strains. Competition studies performed in soil microcosms could help shed light on the outcome of competitive interactions in closely controlled spatially homogeneous and heterogeneous environments.

ACKNOWLEDGMENTS

This research was supported by the University of Idaho.

We thank G. Maria Schneider for help with rep-PCR and data analysis.

REFERENCES

1. **Aakre, R. K., A. Jenkins, B. E. Kristiansen, and L. O. Froholm.** 1998. Clonal distribution of invasive *Neisseria meningitidis* isolates from the Norwegian county of Telemark, 1987 to 1995. J. Clin. Microbiol. **36:**2623–2628.
2. **Cho, J. C., and J. M. Tiedje.** 2000. Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. Appl. Environ. Microbiol. **66:** 5448–5456.
3. **Dombek, P. E., L. K. Johnson, S. T. Zimmerley, and M. J. Sadowsky.** 2000. Use of repetitive DNA sequences and the PCR To differentiate *Escherichia coli* isolates from human and animal sources. Appl. Environ. Microbiol. **66:**2572–2577.
4. **Fulthorpe, R. R., A. N. Rhodes, and J. M. Tiedje.** 1998. High levels of endemicity of 3-chlorobenzoate-degrading soil bacteria. Appl. Environ. Microbiol. **64:**1620–1627.
5. **Grundmann, G. L., and P. Normand.** 2000. Microscale diversity of the genus *Nitrobacter* in soil on the basis of analysis of genes encoding rRNA. Appl. Environ. Microbiol. **66:**4543–4546.
6. **Harwood, C. S., and J. Gibson.** 1988. Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodopseudomonas palustris.* Appl. Environ. Microbiol. **54:**712–717.
7. **Johnsen, K., S. Andersen, and C. S. Jacobsen.** 1996. Phenotypic and genotypic characterization of phenanthrene-degrading fluorescent *Pseudomonas* biovars. Appl. Environ. Microbiol. **62:**3818–3825.
8. **Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap.** 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Appl. Environ. Microbiol. **68:**1854–1863.
9. **Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney.** 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. **63:**4516–4522.
10. **Louws, F., J. Rademaker, and F. de Bruijn.** 1999. The three D's of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. Annu. Rev. Phytopathol. **37:**81–125.
11. **Maynard-Smith, J., N. H. Smith, M. O'Rourke, and B. G. Spratt.** 1993. How clonal are bacteria? Proc. Natl. Acad. Sci. USA **90:**4384–4388.
12. **McArthur, J. V., D. A. Kovacic, and M. H. Smith.** 1988. Genetic diversity in natural populations of a soil bacterium across a landscape gradient. Proc. Natl. Acad. Sci. USA **85:**9621–9624.
13. **Musser, J. M., P. M. Schlievert, A. W. Chow, P. Ewan, B. N. Kreiswirth, V. T. Rosdahl, A. S. Naidu, W. Witte, and R. K. Selander.** 1990. A single clone of *Staphylococcus aureus* causes the majority of cases of toxic shock syndrome. Proc. Natl. Acad. Sci. USA **87:**225–229.
14. **Musser, J. M., J. S. Kroll, D. M. Granoff, E. R. Moxon, B. R. Brodeur, J. Campos, H. Dabernat, W. Frederiksen, J. Hamel, and G. Hammond.** 1990. Global genetic structure and molecular epidemiology of encapsulated *Haemophilus influenzae.* Rev. Infect. Dis. **12:**75–111.
15. **Ochman, H., and R. K. Selander.** 1984. Evidence for clonal population structure in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **81:**198–201.

15a. **Oda, Y., B. Star, L. A. Huisman, J. C. Gottschal, and L. J. Forney.** 2003. Biogeography of the purple nonsulfur bacterium *Rhodopseudomonas palustris*. Appl. Environ. Microbiol. **69:**5186–5191.

16. **Oda, Y., W. Wanders, L. A. Huisman, W. G. Meijer, J. C. Gottschal, and L. J. Forney.** 2002. Genotypic and phenotypic diversity within species of purple nonsulfur bacteria isolated from aquatic sediments. Appl. Environ. Microbiol. **68:**3467–3477.

16a. **Ott, R. L., and M. Longnecker.** 2001. Statistical methods and data analysis. Duxbury, Pacific Grove, Calif.

17. **Palmer, K. M., and J. P. W. Young.** 2000. Higher diversity of *Rhizobium leguminosarum* biovar viciae populations in arable soils than in grass soils. Appl. Environ. Microbiol. **66:**2445–2450.
18. **Purkhold, U., A. Pommerening-Roser, S. Juretschko, M. C. Schmid, H. P. Koops, and M. Wagner.** 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. Appl. Environ. Microbiol. **66:** 5368–5382.
19. **Rademaker, J. L. W., F. J. Louws, and F. J. de Bruijn.** 1997. Characterization of the diversity of ecologically important microbes by rep-PCR genomic fingerprinting, p. 1–26. In A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology manual, supplement 3. Kluwer Academic Publishers, Dordrecht, The Netherlands.
20. **Rius, N., C. Fusté, C. Guasp, J. Lalucat, and J. G. Lorén.** 2001. Clonal population structure of *Pseudomonas stutzeri*, a species with exceptional genetic diversity. J. Bacteriol. **183:**736–744.
21. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Sasikala, C., and C. V. Ramana.** 1998. Biodegradation and metabolism of unusual carbon compounds by anoxygenic phototrophic bacteria. Adv. Microb. Physiol. **39:**339–377.
23. **Scala, D. J., and L. J. Kerkhof.** 2000. Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. Appl. Environ. Microbiol. **66:**1980–1986.
24. **Scala, D. J., and L. J. Kerkhof.** 1999. Diversity of nitrous oxide reductase (*nosZ*) genes in continental shelf sediments. Appl. Environ. Microbiol. **65:** 1681–1687.
25. **Souza, V., M. Rocha, A. Valera, and L. E. Eguiarte.** 1999. Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. Appl. Environ. Microbiol. **65:**3373–3385.
26. **van der Woude, B. J., M. de Boer, N. M. van der Put, F. M. van der Geld, R. A. Prins, and J. C. Gottschal.** 1994. Anaerobic degradation of halogenated benzoic acids by photoheterotrophic bacteria. FEMS Microbiol. Lett. **119:**199–207.
27. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
28. **Whitby, C. B., J. R. Saunders, J. Rodriguez, R. W. Pickup, and A. McCarthy.** 1999. Phylogenetic differentiation of two closely related *Nitrosomonas* spp. that inhabit different sediment environments in an oligotrophic freshwater lake. Appl. Environ. Microbiol. **65:**4855–4862.
29. **Wise, M. G., L. J. Shimkets, and J. V. McArthur.** 1995. Genetic structure of a lotic population of *Burkolderia* (*Pseudomonas*) *cepacia.* Appl. Environ. Microbiol. **61:**1791–1798.
30. **Zhou, J., B. Xia, D. S. Treves, L. Y. Wu, T. L. Marsh, R. V. O'Neill, A. V. Palumbo, and J. M. Tiedje.** 2002. Spatial and resource factors influencing high microbial diversity in soil. Appl. Environ. Microbiol. **68:**326–334.