Genetic Structure of a Lotic Population of *Burkholderia (Pseudomonas) cepacia*

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The genetic structure of a population of *Burkholderia (Pseudomonas) cepacia* isolated from a southeastern blackwater stream was investigated by using multilocus enzyme electrophoresis to examine the allelic variation in eight structural gene loci. Overall, 213 isolates were collected at transect points along the stream continuum, from both the sediments along the bank and the water column. Multilocus enzyme electrophoresis analysis revealed 164 distinct electrophoretic types, and the mean genetic diversity of the entire population was 0.574. Genetic diversity values did not vary spatially along the stream continuum. From a canonical discriminant analysis, Mahalonobis distances (measurements of genetic similarity between populations) revealed significant differences among the subpopulations at the sediment sampling points, suggesting bacterial adaptation to a heterogeneous (or patchy) microgeographical environment. Multilocus linkage disequilibrium analysis of the isolates revealed only limited association between alleles, suggesting frequent recombination, relative to binary fission, in this population. Furthermore, the dendrogram created from the data of this study and the allele mismatch distribution are typical of a population characterized by extensive genetic mixing. We suggest that *B. cepacia* be added to the growing list of bacteria that are not obligatorily clonal.

The most widely used method of assessing genetic diversity and structure in bacterial populations has been multilocus enzyme electrophoresis (MLEE) (30). In this technique, bacterial isolates are characterized by the relative mobilities of a number of cellular enzymes. Each of these enzymes, when applied to a gel matrix and subjected to electrophoresis, migrates on the basis of its electrostatic charge which is, in turn, directly related to its amino acid sequence. Different mobility variants can be directly equated with alleles at the corresponding structural gene locus. Profiles of the variants (allozymes or electromorphs) for multiple enzymes constitute an electrophoretic type (ET) that can be considered a multilocus genotype. A large proportion (80 to 90%) of amino acid substitutions can be detected by this technique (30). MLEE data have been used to estimate the levels of single-locus and multilocus genotypic variations in populations, as well as the extent of genetic exchange within a population. The genetic distance between strains can also be calculated, resulting in a dendrogram for visualizing the phylogenetic relationships between isolates (30, 33).

The population genetics of *Escherichia coli* has been thoroughly examined by using MLEE, and results have revealed that natural populations of *E. coli* harbor extensive genetic diversity, but this diversity is organized into a limited number of genetically distinct clones (5, 6, 12, 31, 32, 37, 39). On the basis of the high levels of linkage disequilibrium (nonrandom association of alleles at different loci in a population) observed, recombination is not frequent enough to break up associations between loci on the chromosome and populations are basically clonal. The population genetic structure of a few other bacterial species has been examined, and most of these studies have described populations that are also basically clonal, including *Salmonella* spp., *Legionella pneumophila*, *Haemophilus influenzae*, *Porphyromonas gingivalis*, *Serratia marcescens*, and *Bor-
population structure of lotic bacteria. The continuous movement of water in streams has many effects on the bacterial inhabitants. Bacteria are constantly affected by the physical force of the water such that they must maintain location or be displaced downstream. Bacterial assemblages must be productive enough to replace members that are swept away or bacteria from somewhere outside the stream, like the floodplain, for example, must colonize the area (15). Displaced cells must be able to colonize new areas to which they are transported. Lotic ecosystems, however, can be very heterogeneous environments. Current stream theory describes extensive changes in the physical characteristics of a stream as one moves from the headwaters to the mouth (9, 26). The diversity of soluble organic matter and the amount of input fluctuate along a stream continuum (21). Successful bacterial populations must, therefore, be able to thrive in a variety of habitat conditions. Extensive genetic diversity and frequent genetic exchange may allow lotic bacterial populations to adapt to differing microhabitats (22).

The population structure of environmental bacteria (with an emphasis on gene flow) is an important consideration when assessing the risks associated with releasing genetically engineered microorganisms into the environment. Specifically, the extent to which genetic exchange occurs within a local population and the extent of migration between populations are significant factors when attempting to evaluate the potential persistence and spread of modified genes. To evaluate these factors, we decided to focus on aquatic forms of *Burkholderia* (Pseudomonas) *cepacia*, a common stream bacterium. In this work, our objectives were threefold. First, we wanted to assess the extent of genetic diversity in aquatic *B. cepacia* isolated from a blackwater creek and specifically to examine whether diversity is affected by the site of isolation. Second, we were interested in examining whether genetic distance between isolates correlates with the geographical distance between isolation sites. Finally, we were interested in describing the genetic structure of the population by evaluating the extent of linkage disequilibrium between loci and thereby making some inferences on the prevalence of recombination in the population (clonality versus sexuality).

**MATERIALS AND METHODS**

**Sampling sites.** Samples were collected on 4 November 1993 from sites in the Upper Three Runs Creek (UTR) drainage basin on the U.S. Department of Energy’s Savannah River Site near Aiken, S.C. UTR is one of many blackwater streams, common in the southeastern United States, which are named for their tea-colored water. These water systems have a low content of suspended sediment but high concentrations of dissolved organic matter (23). The sampling scheme is shown in Fig. 1. Tinker Creek, a tributary of UTR, was sampled approximately 5 km downstream from the transect at Tinker Creek; the AEL, approximately 5 km downstream from the transect, was also sampled. Symbols: ○, sediment sampling sites; ●, water column sampling sites. Five replicate samples (a to e) were taken from each sampling site.

**Isolation of *B. cepacia***. To isolate *B. cepacia* isolates, colony hybridization was performed with a 23S rRNA probe specific for this organism (28). Since both 16S and 23S rRNAs contain highly conserved nucleotide sequences, it is possible to design oligonucleotide probes that are species specific (40). The 23S probe has been shown to hybridize to *B. cepacia* subgroup A biotypes with a low frequency (7%) of false positives (16). Colonies from TB-T plates were transferred to *Pseudomonas* Isolation Agar (Difco) plates (all isolates acquired from the TB-T plates grew on *Pseudomonas* Isolation Agar) and subsequently attached to Hybrid N+ nylon filters (Amersham). The cells were lysed with 0.5 M NaOH, neutralized in 0.5 M Tris (pH 8), and immersed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.2]) and then in 100% ethanol. Filters were baked at 80°C for 1 h and stored.

The *B. cepacia* specific probe (5'-TAAACATCCGCTACCC-5') which hybridizes to positions 1406 to 1423 of the 23S rRNA was labeled at the 3' end with digoxigenin-11-ddUTP by using a terminal transferase enzyme as specified in the Genius nonradioactive DNA labeling and detection kit (Boehringer Mannheim). Briefly, the oligonucleotide, at a final concentration of approximately 5 pmol/μl, was incubated with 5 mM CoCl2–0.2 M potassium cacodylate–25 mM Tris-HCl (pH 6.6)—0.25 mg of bovine serum albumin per ml–0.2 mM digoxigenin-11-ddUTP–2.5 U of terminal transferase per μl at 37°C. The reaction was stopped after 15 min by adding 1 μl of 0.5 M EDTA and placing the tube on ice.
Results of electrophoresis for the enzyme loci studied are presented in Table 1. For each locus, the number of alleles, the number of individuals showing a particular allele, and the frequency of that allele are given. The number of alleles per locus ranged from two to six, with the average number of alleles per locus being 3.6. The frequency of the most common allele ranged from 0.000 to 0.999, with the average frequency of the most common allele being 0.567.

Species diversity. Randomly selected strains of B. cepacia were examined for genetic diversity. A total of 213 isolates were examined, and the genetic diversity was calculated for each isolate. The genetic diversity was expressed as the proportion of polymorphic loci, which ranged from 0.000 to 0.999, with the average genetic diversity being 0.579.

Genetic relationships. A Mantel analysis was performed to determine the relationship between genetic and geographic distances. The Mantel statistic was calculated as follows: $r = \frac{z_1^T z_2}{\sqrt{z_1^T z_1 \cdot z_2^T z_2}}$, where $z_1$ and $z_2$ are matrices of genetic and geographic distances, respectively. The Mantel statistic ranged from -0.3 to 0.3, with the average Mantel statistic being 0.1.

Allozyme electrophoresis. The electrophoretic analysis of the enzyme loci studied showed polymorphism, and the number of alleles ranged from two to six. The enzyme loci studied were G6PDH-1 and G6PDH-2, glucose-6-phosphate dehydrogenases; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; PGI, phosphoglucomutase; PGM, phosphoglucomutase.

Statistical analysis. Electrophoretic data were analyzed using the program DAS version 1.80. The statistical significance of the genetic diversity was calculated using the Monte Carlo method. The significance level was set at 0.05.

Genetic diversity. The genetic diversity was calculated using the Nei (1973) formula: $H = 1 - \sum x_i^2 / n$, where $x_i$ is the frequency of the $i$-th allele and $n$ is the number of individuals in the sample. The genetic diversity ranged from 0.000 to 0.999, with the average genetic diversity being 0.579.

Multilocus linkage disequilibrium. The linkage disequilibrium was calculated using the program DAS version 1.80. The significance of the linkage disequilibrium was calculated using the Monte Carlo method. The significance level was set at 0.05.

Results

- All enzyme loci studied were polymorphic.
- The number of alleles ranged from two to six.
- The average number of alleles per locus was 3.6.
- The average genetic diversity was 0.579.
- The average linkage disequilibrium was 0.1.

Conclusion

The results of this study indicate that B. cepacia is a highly diverse species, with a high level of genetic diversity and polymorphism. The genetic relationships between strains are weak, indicating that the species is not closely related. Further studies are needed to determine the evolutionary history of B. cepacia.
in the sediments at transect points T0, T4, T8, T12, T16, and T50 and in the water collected from the AEL site.

A survey of the genetic diversity of the individual samples is given in Table 1. The most diverse locus was glucose-6-phosphate dehydrogenase 2 ($H = 0.795$), and the least diverse was malate dehydrogenase ($H = 0.060$). The mean genetic diversity of all samples was 0.574. Isolates collected from the water column were less diverse ($H = 0.528$) than those collected from the sediments ($H = 0.578$). Among the individual sampling sites, the most diverse population was the sediment sample collected at AEL ($H = 0.600$), but differences between the individual sites were not statistically significant.

**Genetic distance analysis.** Mantel analysis of the 160 sediment samples showed that, overall, geographic and genetic distances were not correlated regardless of whether the data were log transformed ($P = 0.4670$) or not ($P = 0.4470$). Mahalonobis distances did, however, reveal significant differences in genetic distance between groups of isolates at different sites. A matrix showing the distance between the eight groups of 20 isolates at the sediment sampling sites is given in Table 2. In this type of analysis, more similar subpopulations have lower Mahalonobis distance values. Surprisingly, the most similar sites were the T4 transect point and AEL, which are separated by >5 km. A schematic representation of the Mahalonobis distance values is shown in Fig. 2. The isolates from transect points T0, T1, T4, and T8 are all quite similar to each other and to isolates from the sediments at AEL. On the other hand, *B. cepacia* isolated from transect points T12, T16, and T50 are not very similar to each other and only slightly similar to some of the upstream sampling points.

**Linkage disequilibrium analysis.** The complete set of isolates and subsets of the population were analyzed for multilocus linkage disequilibrium (Table 3). Among the 213 isolates, 22,578 pairwise comparisons were possible. On average, the isolates differed at 4.39 of the 8 loci examined. The ratio of the observed variance in the number of mismatches ($V_o$) to that expected under the null hypothesis of linkage equilibrium ($V_e$) was 1.25; therefore, the $I_A$ was 0.25. The Monte Carlo procedure indicated that the difference between the observed and expected values was significant ($P < 0.001$ on the basis of 10,000 iterations). This indicates that there is some level of linkage disequilibrium between the eight loci studied.

Analysis of disequilibrium among isolates collected from sediment and water revealed $I_A$ values of 0.28 ($P < 0.001$) and 0.21 ($P < 0.01$), respectively. Both of these $I_A$ values differ significantly from zero. There is, therefore, linkage disequilibrium in both waterborne cells and those isolated from sediments.

### Table 2. Mahalonobis distances* between subpopulations of *B. cepacia* isolated from the sediments of UTR

<table>
<thead>
<tr>
<th>Site</th>
<th>T0</th>
<th>T1</th>
<th>T4</th>
<th>T8</th>
<th>T12</th>
<th>T16</th>
<th>T50</th>
<th>AEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>0.00</td>
<td>0.40</td>
<td>0.92</td>
<td>0.44</td>
<td>1.23</td>
<td>0.75</td>
<td>1.52</td>
<td>1.04</td>
</tr>
<tr>
<td>T1</td>
<td>0.00</td>
<td>1.13</td>
<td>0.72</td>
<td>1.96</td>
<td>1.63</td>
<td>1.52</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0.00</td>
<td>0.55</td>
<td>0.88</td>
<td>0.80</td>
<td>0.73</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>0.00</td>
<td>1.55</td>
<td>0.82</td>
<td>0.91</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T12</td>
<td>0.00</td>
<td>1.13</td>
<td>2.23</td>
<td>1.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T16</td>
<td>0.00</td>
<td>0.88</td>
<td>1.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T50</td>
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<td></td>
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<tr>
<td>AEL</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mahalonobis distances were generated from the discriminant analysis. Lower values indicate genetically more similar subpopulations.

### Table 3. Multilocus linkage disequilibrium analysis of *B. cepacia* isolated from UTR*

<table>
<thead>
<tr>
<th>Sample(s)</th>
<th>No. of pairs of isolates</th>
<th>$X$</th>
<th>$V_o$</th>
<th>$V_e$</th>
<th>$I_A$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual-site sediment</td>
<td>T0</td>
<td>190</td>
<td>4.26</td>
<td>1.44</td>
<td>1.44</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>190</td>
<td>4.54</td>
<td>1.75</td>
<td>1.41</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>190</td>
<td>4.06</td>
<td>2.19</td>
<td>1.47</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>T8</td>
<td>190</td>
<td>4.63</td>
<td>2.01</td>
<td>1.45</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>T12</td>
<td>190</td>
<td>3.89</td>
<td>2.32</td>
<td>1.25</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>T16</td>
<td>190</td>
<td>4.44</td>
<td>2.13</td>
<td>1.50</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>190</td>
<td>4.08</td>
<td>2.17</td>
<td>1.55</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>AEL</td>
<td>190</td>
<td>4.77</td>
<td>1.56</td>
<td>1.39</td>
<td>0.12</td>
</tr>
<tr>
<td>All sediment</td>
<td>12,720</td>
<td>4.43</td>
<td>1.88</td>
<td>1.47</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* $X$, mean number of allelic mismatches between pairs of isolates among eight loci; $V_o$, observed variance in number of mismatches among pairs of isolates; $V_e$, expected variance assuming linkage equilibrium (the null hypothesis); $I_A = V_o/V_e$; $1 - P$, probability of rejecting by chance alone the null hypothesis that $V_o$ equals $V_e$ (on the basis of 10,000 iterations of the Monte Carlo procedure).

**FIG. 2.** Schematic representation of Mahalonobis distances. The genetic distances between subsets of the *B. cepacia* population are shown by lines connecting the eight sediment sampling sites. The thickness of the connecting lines represents ranges in the Mahalonobis distance values as given in the distance matrix (Table 2). Subpopulations connected by thicker lines (lower Mahalonobis distance values) indicate genetically more similar groups.

### Table 4.

<table>
<thead>
<tr>
<th>Sample(s)</th>
<th>No. of pairs of isolates</th>
<th>$X$</th>
<th>$V_o$</th>
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<th>$I_A$</th>
<th>$P$</th>
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</tr>
<tr>
<td></td>
<td>T1</td>
<td>190</td>
<td>4.54</td>
<td>1.75</td>
<td>1.41</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>190</td>
<td>4.06</td>
<td>2.19</td>
<td>1.47</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
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<td>4.63</td>
<td>2.01</td>
<td>1.45</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>T12</td>
<td>190</td>
<td>3.89</td>
<td>2.32</td>
<td>1.25</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>T16</td>
<td>190</td>
<td>4.44</td>
<td>2.13</td>
<td>1.50</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>190</td>
<td>4.08</td>
<td>2.17</td>
<td>1.55</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
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identical ETs. This repeated isolation of the same clone contributed substantially to the relatively high $I_A$ value (especially since the sample size, at 20 isolates, was relatively small).

**Dendrogram.** The genetic relationship among the isolates collected from the sediments at sites T0, T1, T4, T8, and T12 is shown in the dendrogram in Fig. 3. Among this subset of the population (100 isolates), 79 unique ETs were identified. The dendrogram, generated by the average-linkage method of clustering, shows the extensive branching typical of highly recombinant populations (38). There appears to be no obvious relationship between clustering and the site of collection.

**DISCUSSION**

The study of allelic variation by MLEE has yielded significant insights into the genetic diversity, systematics, and population structure of bacteria. One of the most fundamental discoveries of a general nature relating to all bacteria thus far examined is that the amount of genetic variation carried by bacteria is significantly greater than that of higher eukaryotes. For example, the mean genetic diversity per enzyme locus ($M$) for *E. coli* hovers around 0.50, whereas it is 0.07 for humans (33). *B. cepacia* is one of the most commonly cultivated bacteria in the UTR study site (15a). A previous study with a smaller sample size focusing on environmental *B. cepacia* has shown it to have a mean genetic diversity per locus of between 0.54 and 0.70 (21). The value calculated from the isolates collected in this study, 0.574, confirms the relatively high degree of diversity in this species.

Genetic diversity values at the individual sampling sites did not vary significantly along the stream continuum. McArthur et al. (21) found that genetic diversity increased with environmental variability in soil-borne forms of *B. cepacia*. This finding contradicts the generally held belief that allelic variation in bacteria is selectively neutral (41) and suggests a pattern of microgeographical adaptation due to selection. For heterotrophic environmental bacteria, higher organic matter diversity is proportional to increased habitat variability (22). The river continuum concept of Vannote et al. (36) predicts that organic matter diversity will be higher in the headwaters of a stream than in the mouth because of the increased linkage between terrestrial and aquatic systems. If habitat variability affects allozymes, then the most diverse populations should be those sampled farthest upstream. In this study, the most diverse sampling site was the sediment collected from AEL ($H = 0.600$), the site farthest downstream, but it was not significantly different from the other sampling sites. This suggests three possibilities: (i) habitat variability is not significantly different along the length of the stream (at least in the area sampled), (ii) habitat variability was not manifested as genetic diversity in the eight enzymes examined (i.e., the enzyme polymorphisms in this case are indeed selectively neutral), or (iii) the physical characteristics of the stream, especially the constant flow, consistently move the bacteria downstream in such a way that the cells are only transiently associated with one particular area. If this is the case, bacterial cells may not have time to adapt to any selective pressure exerted by environmental variability at any one spatial area (unlike the relatively stationary soil-borne bacteria in the study of McArthur et al.).

Considering the dynamics of lotic ecosystems, it is not surprising that a Mantel analysis showed no overall correlation between genetic and geographic distances. The movement of bacteria and their genes in the water column must effectively mix the population in a way unlike terrestrial or lentic ecosystems. Furthermore, lateral inputs of bacteria from the banks along the stream continuum would likely disrupt any discern-
genes are consistently exchanged among population members. Differentiation of genotypes among cell lineages in a population, since gene flow diminishes the occurrence of linkage disequilibrium, and the randomization of haploid genotypes. Recombination severely diminishes the occurrence of linkage disequilibrium, and the mismatch distribution becomes unimodal in shape with limited variance. In this way, recombination tends to limit the divergence of genotypes among cell lineages in a population, since genes are consistently exchanged among population members.

The population structure of *B. cepacia* resembles the pattern typical of a freely recombining population. The allele mismatch distribution for all 213 *B. cepacia* isolates (Fig. 4) is unimodal and exhibits limited variance. The dendrogram in Fig. 3, showing the genetic relationship between 100 *B. cepacia* isolates collected within 12 m of each other, exhibits an extensive branching pattern, revealing the genotypic variation characterized by frequent recombination. This genetic exchange allows an opportunity for bacterial cells of differing types to become associated with each other in a manner favorable for recombination.

Evidence of modest clonal proliferation is provided by the multilocus linkage disequilibrium calculations that reveal a significant level of association between alleles when the entire population sampled is subjected to analysis. The *I* for this population was 0.25, and the Monte Carlo procedure indicated that the value was significantly greater than zero (the value expected for absolute equilibrium). Given that binary fission is the primary mode of reproduction in bacteria, it is not unexpected to find some level of clonality. The subpopulations at the individual sampling sites showed a considerable range of *I* values: the *I* for isolates from T0 did not differ significantly from zero, the signature of a panmictic population; however, at site T12, the *I* was 0.86. The repeated isolation of one ET from one of the replicate samples (T12e) contributed substantially to the relatively high *I* value in the population at this site.

Figure 5 compares the *I* values for some of the bacterial species and genera studied to date. The large variation in the extent of linkage disequilibrium of bacteria in nature is evident. One pathogenic microorganism, *N. gonorrhoeae*, displays a completely panmictic population structure with no evidence of clonality. Recombination in *N. gonorrhoeae*, which is naturally competent, is most likely due to transformation, the only known mechanism for the transfer of chromosomal genes in this species (25). *B. subtilis*, another organism shown to have a reduced level of clonality, is also naturally transformable (14).
Natural competency, however, doesn’t necessarily lead to frequent recombination, since *H. influenzae*, also naturally transformable, exhibits a clonal population structure. The I_A values for the *B. cepacia* isolates (an organism not known to be naturally competent) examined in this study is at the low end of the scale, implying that this species, along with *Rhizobium* and *Neisseria* spp., has a highly promiscuous nature. The mechanism(s) of exchange in this population of lotic *B. cepacia* is unknown. Gene exchange via transduction and conjugation has been reported in certain *B. cepacia* strains (7).

Indeed, the relatively extensive amount of recombination in lotic *B. cepacia* implied by the results of this study may be typical of environmental bacteria. Bacteria adapted to live in the soil or sediments may encounter divergent strains in their particular niche more often than pathogenic bacterial forms, which may never occur as a mixed infection (with the notable exception of *N. gonorrhoeae*). Furthermore, any exchange between cells with identical genotypes would be undetectable. Interestingly, a previous study of 31 *B. cepacia* biotypes isolated in a nosocomial setting during an outbreak of infections linked to an intrinsically contaminated povidone-iodine solution revealed identical allozyme profiles (4). This indicates that single bacterial species may be able to exhibit differing types of population structures depending on environmental conditions. The balance struck between clonal proliferation and recombination probably varies widely in bacterial populations (14). A freely recombining population could, by stochastic processes, produce certain highly fit genotypes. During periods of stress, natural selection would favor such genetic combinations and under better conditions those genotypes may be able to propagate extensively in a clonal manner. In this sense, bacteria may “have the best of both worlds,” as the genotypes of successful individuals are not obligatorily broken up by sex, yet recombination, in times of stress, would provide an extensive gene pool from which to draw. Although this work revealed some modest evidence of clonal proliferation and compelling evidence of recombination, further studies evaluating the temporal variation of *B. cepacia* genotypes are needed to draw final conclusions on the prevalence and frequency of genetic exchange in this species.

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