Epidemiology of *Burkholderia cepacia* Infection in Patients with Cystic Fibrosis: Analysis by Randomly Amplified Polymorphic DNA Fingerprinting

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Received 23 May 1996/Returned for modification 5 August 1996/Accepted 4 September 1996

We fingerprinted a collection of 627 *Burkholderia cepacia* isolates from 255 patients with cystic fibrosis (CF) and 43 patients without CF and from the environment, by a PCR-based randomly amplified polymorphic DNA (RAPD) method with primers selected for their ability to produce discriminatory polymorphisms. The RAPD typing method was found to be reproducible and discriminatory, more sensitive than PCR ribotyping, and able to group epidemiologically related *B. cepacia* strains previously typed by both pulsed-field gel electrophoresis and conventional ribotyping. Seven strain types infecting multiple CF patients were found at several different CF treatment centers in Canada, the United States, the United Kingdom, France, and Australia, indicating the presence of epidemic strain types. Most CF patients were each colonized with a single strain type, and several patients harbored the same strain type for 5 or more years. *B. cepacia* isolates recovered from other clinical sources (525 isolates examined) and from the environment (58 isolates examined) possessed RAPD fingerprints that were generally distinct from CF-associated strain types (525 isolates examined). RAPD is a versatile fingerprinting method for studying the epidemiology of *B. cepacia*.

Cystic fibrosis (CF) is the most common potentially fatal autosomal recessive disease in North America, affecting approximately 1 in 2,000 live births among Caucasians (5). Although *Pseudomonas aeruginosa* is the predominant respiratory pathogen in patients with CF (26), *Burkholderia* (formerly *Pseudomonas*) *cepacia* has emerged as a particularly problematic pulmonary pathogen in these patients. The organism is highly virulent in certain patients with CF (8, 30). It is intrinsically resistant to a wide range of antimicrobial agents (22), and there is considerable evidence that *B. cepacia*, unlike *P. aeruginosa*, can spread from one CF patient to another both within and outside the hospital (2, 7, 9, 13, 15). However, investigations finding no evidence of patient-to-patient transmission (27) or documenting only the transmission of one epidemic clone (29) suggest that the transmissibility of *B. cepacia* may vary depending on a number of factors including strain, treatment center, and CF patient population. It has become critically important to determine the risk factors for patient-to-patient spread of *B. cepacia* and to identify strains that are prevalent and pose the greatest risk of infection at CF treatment centers.

Several techniques have been employed for typing *B. cepacia*. Phenotypic methods such as serology, biochemical profile, or pigment production have been widely used (23) but are subject to instability because the phenotype of *B. cepacia* CF isolates may vary markedly (12). Multilocus enzyme electrophoresis, a phenotypic method, has also been applied to *B. cepacia* (9); multilocus enzyme electrophoresis demonstrated that CF isolates are clustered clonally and are generally distinct from nosocomial and environmental isolates; however, the number of strains examined from non-CF sources was small. Genetic typing methods, such as ribotyping (28), have been shown to provide good specificity and sensitivity for epidemiological study of *B. cepacia* (23). Ribotyping analysis has been used to demonstrate patient-to-patient transmission (7, 13, 25), clustering of *B. cepacia* strains at treatment centers (15), and transatlantic spread of one transmissible lineage (9, 29). Typing by pulsed-field gel electrophoresis (PFGE) has also demonstrated the spread of certain strains among CF patients (7, 25, 29); however, Steinbach et al. (27) reported no PFGE typing evidence for person-to-person transmission of *B. cepacia* at one CF treatment center. Three PCR-based methods for typing *B. cepacia* have also been reported: PCR ribotyping (11), randomly amplified polymorphic DNA (AP-PCR or RAPD) (2), and enterobacterial repetitive intergenic consensus sequence PCR (17).

Over the last 10 years, our laboratory has established a large collection of *B. cepacia* isolates from pediatric and adult CF treatment centers in Vancouver, British Columbia, Canada. This collection has recently been expanded to include isolates from other CF centers in Canada, the United States, the United Kingdom, France, and Australia, as well as many isolates from other clinical sources and from the environment. We sought to type this collection of isolates to establish which (if any) strains of *B. cepacia* are prevalent in patients with CF at various treatment centers and to identify strains that might be transmitted from patient to patient. Because of the large numbers of isolates involved, a PCR-based assay was utilized to enable high sample throughput (10, 18). We developed a RAPD typing method, which, unlike that reported previously (2), utilized several different arbitrary primers screened and selected for their ability to produce stable and discriminatory polymorphisms. The same RAPD method has been successfully applied to differentiate a large collection of *P. aeruginosa* isolates from CF patients (18). The RAPD typing results of 627
B. cepacia isolates from patients at several CF treatment centers and from various other clinical and environmental sources form the basis of this report.

MATERIALS AND METHODS

Collection of B. cepacia isolates and microbiological methods. Isolates of B. cepacia were received from CF clinics and clinical and research laboratories across Canada and from the United States and the United Kingdom. A total of 627 isolates were examined and included 525 isolates recovered from 255 patients with CF, 44 isolates recovered from 43 patients without CF, and 58 isolates extracted from B. cepacia frozen culture stock. Primers numbers are indicated above each lane. (B) The effect of template DNA concentration on the RAPD fingerprint profile. The amount of DNA (nanograms) added to each reaction mixture is shown above each lane; DNA extracted from B. cepacia frozen culture stock. Primers numbers are indicated above each lane.

RESULTS

Identification of primers for RAPD analysis. The polymorphisms amplified by the eight functional RAPD primers from DNA extracted from two subcultures of B. cepacia ATCC 25416 made from the same freezer vial on two separate occasions are shown in Fig. 1A. Each primer amplified a DNA fingerprint ranging from 5 to 20 bands, over a size range of 100 bp to 5 kb, which was reproducible for the two independent preparations of DNA. Two conditions were found to affect the reproducibility of the RAPD fingerprint: template DNA concentration and primer concentration. The effect of the template DNA concentration on fingerprint profile is shown in Fig. 1B. The RAPD polymorphisms remained stable with 10 to 100 ng of template DNA; if less than 10 ng of B. cepacia DNA was present in the PCR, no or partial fingerprints were obtained, and with greater than 100 ng of DNA, there was loss of banding (Fig. 1B). RAPD fingerprints were also stable when between 10 and 160 pmol of primer was added to reaction mixtures containing 40 ng of template DNA (data not shown). Reaction mixtures containing 40 ng of template DNA and 40 pmol of RAPD primer were found to be optimal for amplification of reproducible fingerprints from B. cepacia and were applied throughout the study. Failure to generate a reproducible fingerprint from a sample of DNA was rare; such problems were corrected by preparation of a fresh DNA stock, and reproducible fingerprints were generated for all 627 isolates examined in the study. Although all eight primers were found to discriminate among unrelated B. cepacia strains (data not shown), primer 270 (the first primer to be evaluated) was used to type all the isolates examined in this study (see below). Primers 208 and 272 were used to confirm RAPD types found with primer 270; further evaluation of the remaining five primers was not carried out.

Identification of genetically related B. cepacia strains. All B. cepacia isolates were typed without prior knowledge of their source by RAPD with primer 270. Nine groups of related RAPD fingerprints and five unique fingerprints were found in
the first 60 isolates examined. The polymorphisms generated with primer 270 of these nine RAPD types are shown in Fig. 2A. The fingerprints generated by primer 270 enabled good primary discrimination of B. cepacia strain types. Within RAPD types, the amplified polymorphisms were conserved and the similarity coefficients of the banding profile were above 0.7 as determined by computer-assisted analysis. Between RAPD types, differences in the number of amplified markers, their molecular size, and intensity were considerable and enabled good interstrain discrimination.

The RAPD typing criteria applied to the first 60 isolates also enabled epidemiologically related strains to be grouped, including sequential isolates from individual CF patients and isolates of published epidemiologic and ribotype similarity (see below and Table 1). For example, RAPD types 3, 5, 6, and 7 were representative of sequential isolates each recovered from individual CF patients attending the pediatric clinic in Vancouver (type 6 was later recovered from additional CF patients [see below]). Types 8 and 10 were each representative of sequential isolates recovered from individual CF patents in Seattle, Wash. (12), and Philadelphia, Pa. (14); the published ribotype of each strain type was also distinct (12, 14). Type 1, 2, and 4 strains each infected multiple CF patients but were center related in epidemiology (see below). Isolates belonging to each epidemiologically related cluster possessed RAPD fingerprints with similarity coefficients above 0.7 and differed by no more than three bands; these criteria formed the experimen
tal basis upon which all further isolates were typed by RAPD.

To validate the strain types assigned by RAPD against another PCR typing method, the first 60 isolates were also evaluated by PCR-mediated ribotyping (11). The polymorphisms generated before and after restriction digestion with the endonuclease HaeIII are shown in Fig. 2B and C, respectively. Within each B. cepacia strain type designated by RAPD, isolates also possessed a conserved PCR-ribotype fingerprint (Fig. 2B); however, PCR ribotyping did not easily distinguish among some of the RAPD-assigned types (e.g., groups 1, 4, and 6). The restriction fragment length polymorphisms obtained after digestion of the ribotype products with the enzyme HaeIII are shown in Fig. 2C. The endonuclease-digested PCR-ribotype profiles of members of an RAPD group were also identical. The HaeIII restriction fragment length polymorphism of the amplified PCR-ribotype polymorphisms enabled 8 of the 10 groups to be differentiated, but the polymorphisms generated for groups 4 and 6 remained similar in profile and indistinguishable. All further B. cepacia isolates described in this study were typed by RAPD fingerprinting.

**RAPD analysis of B. cepacia isolates recovered from patients with CF.** The results of the RAPD analysis of B. cepacia isolates recovered from CF patients are summarized in Table 1. A total of 525 CF isolates were analyzed, and 20 fingerprint types in which two or more isolates shared the same pattern were identified; 58 isolates possessed fingerprint profiles which were unique. Ten isolate types, 1, 2, 4, 6, 13, 15, 17, 23, 35, and 40, were recovered from two or more CF patients (Table 1). Type 15 B. cepacia was recovered from two pediatric patients in Vancouver (Table 1); however, the strain was subsequently not cultured from one of the patients, who became stably colonized with type 6 B. cepacia. Type 23 B. cepacia isolates were recovered from two CF patients in Oklahoma, and the type 35 isolates were recovered from two CF patents in Edinburgh; no other strains of each type were present in our collection to support their transmissibility.

Of the remaining B. cepacia types infecting multiple patients, each was recovered from three or more patients. Type 1 B. cepacia was the predominant strain type in the Vancouver pediatric CF clinic and also infected CF patients in the United States and France. Type 2 isolates were recovered from multiple CF patients in the United Kingdom and across Canada; this RAPD type included strains of published ribotype and PFGE fingerprint which belong to the epidemic B. cepacia lineage (7, 15, 29). Type 4 B. cepacia was the predominant strain among adult CF patients in Vancouver and also infected patients in Quebec and Nova Scotia. B. cepacia type 6 was recovered from five CF patients in Vancouver. Type 17 organisms were recovered from a CF patient in Ontario and included strains representative of the predominant B. cepacia ribotype infecting multiple patients at a CF treatment center in Cleveland (15). Finally, type 40 B. cepacia was an epidemic strain type which was recovered from 17 CF patients in an Australian treatment center.

**RAPD group 2** was the most common CF strain type in our collection (267 of 525 CF isolates tested). RAPD analysis demonstrated that this strain type was widespread in Canadian CF clinics, infecting more than 100 CF patients residing in Nova Scotia, Newfoundland, Ontario, Alberta, and British Columbia (Table 1). The polymorphisms amplified by primer 270 from 38 members of this typing group are shown in Fig. 3. Minor variations in fingerprint patterns within RAPD type are illustrated in Fig. 3. The similarity coefficients of the polymorphisms were greater than 0.8, designating them as a single RAPD type. However, the isolates from Newfoundland were
consistently different from the rest, having one extra band of approximately 0.7 kb (Fig. 3). Isolate B491 from the Toronto center was the only other member of B. cepacia type 2 to have this band. There was no difference in the PCR-ribotype polymorphisms of these isolates (Fig. 2B and C).

**Epidemiology of B. cepacia colonization in Vancouver.** A total of 58 CF patients attending clinics in Vancouver were colonized with B. cepacia. The pediatric and adult treatment centers were on the same hospital site separated by about 200 m until September 1993, at which time the adult CF clinic was moved to another hospital. Although the clinics were in proximity, the patients were cared for by different staff, there was little interaction between the clinics, and the patients were hospitalized in separate facilities. The prevalent B. cepacia strain types at each CF clinic were different (Fig. 4). B. cepacia type 1 predominated among patients attending the pediatric clinic (9 of 30 patients); two adult patients were colonized with this strain type. B. cepacia type 4 was the predominant strain among patients attending the adult CF clinic (11 of 18 patients). Two CF patients who were colonized as children with types 5 and 7 subsequently lost these strains and became colonized with type 4 after attending the adult clinic. The epidemiology of RAPD type 2, the epidemic B. cepacia strain (7, 29), in Vancouver was investigated. Although this type was preva-
from Israel, type 24, matched the fingerprint of three *B. cepacia* isolates recovered from a CF patient in Oklahoma.

**DISCUSSION**

The RAPD technique we have developed enabled large numbers of *B. cepacia* isolates from our strain repository to be compared at the genetic level. RAPD was able to distinguish *B. cepacia* isolates more effectively than PCR ribotyping, consistently type serial isolates from individual CF patients, group isolates that were related epidemiologically, and appropriately distinguish isolates with known ribotype and PFGE genomic fingerprints without prior knowledge of epidemiology. Our study also provides further evidence that CF treatment centers may harbor one or more predominant strains of *B. cepacia* and that these CF isolates are, in general, genetically distinct from other clinical isolates and environmental strains.

RAPD fingerprinting was able to produce a discriminatory and reproducible genetic fingerprint from all *B. cepacia* isolates tested. We used this method to examine *P. aeruginosa* isolates recovered from CF patients and found it to be as sensitive as PFGE for typing this species once discriminatory primers were identified (18). For *P. aeruginosa* (18) and *B. cepacia*, the primer-to-template ratio was optimized; however, the PCR cycle conditions described by Akopyanz et al. (1) were unaltered. These data suggest that the original parameters described by Akopyanz et al. (1) for typing *Helicobacter pylori* are a versatile RAPD thermal cycle that may be applied to many bacterial species. Indeed, reproducible and discriminatory polymorphisms were amplified from the following bacterial species that were tested as part of our collection because they had been originally misidentified as *B. cepacia* (3): *Alcaligenes faecalis, Alcaligenes xylosoxidans, Burkholderia gladioli, Comamonas acidovorans, Enterobacter agglomerans, and Stenotrophomonas (Xanthomonas) maltophilia* (17a). Successful typing of *B. cepacia, P. aeruginosa* (10, 18), and *H. pylori* (1) with the same basic RAPD technique illustrates that such techniques are transferable from one laboratory to another and that reports of unreliable RAPD typing schemes (9) are misleading.

**TABLE 3. RAPD analysis of *B. cepacia* isolates from environmental sources**

<table>
<thead>
<tr>
<th>RAPD type</th>
<th>No. of isolates</th>
<th>Source</th>
<th>Geographic location</th>
<th>Repository no. (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02'</td>
<td>1</td>
<td>Hospital</td>
<td>Cardiff, UK</td>
<td>CEP137</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>Soil</td>
<td>Edinburgh, UK</td>
<td>CEP159</td>
</tr>
<tr>
<td>14'</td>
<td>1</td>
<td>Hospital</td>
<td>Manchester, UK</td>
<td>CEP181</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>Soil</td>
<td>Edinburgh, UK</td>
<td>CEP240</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>Onion</td>
<td>USA</td>
<td>CEP072</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>Onion</td>
<td>USA</td>
<td>CEP073</td>
</tr>
<tr>
<td>24'</td>
<td>1</td>
<td>Hospital</td>
<td>Israel</td>
<td>CEP195</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>Onion</td>
<td>USA</td>
<td>CEP076</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>Hospital</td>
<td>Cardiff, UK</td>
<td>CEP181</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>Soil</td>
<td>UK</td>
<td>CEP155</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>Soil</td>
<td>ATCC 17616</td>
<td>CEP144</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>Soil</td>
<td>ATCC 35130</td>
<td>CEP084</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>Plant/pond</td>
<td>Edinburgh, UK</td>
<td>CEP193 (4)</td>
</tr>
<tr>
<td>37</td>
<td>2</td>
<td>Hospital</td>
<td>Manchester, UK</td>
<td>CEP171</td>
</tr>
<tr>
<td>39'</td>
<td>6</td>
<td>Onion</td>
<td>ATCC 25416</td>
<td>CEP031</td>
</tr>
<tr>
<td>Unique</td>
<td>21</td>
<td>Various</td>
<td>Various</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: UK, United Kingdom; USA, United States.
* Strain number of representative isolate.
* Also a CF RAPD type (see Table 1).
* Also a clinical RAPD type (see Table 2).
R.A.P.D. analysis correctly clustered isolates of known ribotype and PFGE profile (7, 15), although direct comparison with these methods was not performed. Comparison of RAPD fingerprints with PCR-ribotyping profiles demonstrated, as others have described (17), that PCR ribotyping is not as discriminatory as RAPD for epidemiological analysis of B. cepacia. Kostman et al. (11) suggested that restriction digestion of the amplified products of PCR ribotyping may improve the discriminatory power of PCR ribotyping. Digestion of PCR-ribotyping products with the enzyme HaeIII did not significantly improve the discrimination of the B. cepacia isolates examined in this study. RAPD has been shown to be as discriminatory as PFGE (2, 17) and enterobacterial repetitive intergenic consensus sequence PCR (17) for distinguishing B. cepacia; however, day-to-day variation in the fingerprints of the RAPD method evaluated (2) was found (17). Such variation was not apparent with our method, and the good reproducibility was also found with RAPD analysis of P. aeruginosa (18). The large amount of template DNA required for RAPD (a minimum of 10 ng [Fig. 1B]) may also be advantageous for a PCR-based assay, since contaminating DNA would not produce conflicting polymorphisms unless it represented more than 25% of the sample (40 ng was used in each PCR). In contrast, because of the specific nature of ribotyping primers (11), PCR ribotyping was able to amplify polymorphisms from as little as 10 pg of DNA (data not shown), indicating that trace amounts of contaminating DNA may interfere with the banding patterns produced by this PCR method.

Our study provides further evidence that CF centers may harbor one or more predominant B. cepacia strains. Steinbach et al. (27) found no evidence of strain transmission among 17 CF patients at a single treatment center and stated that previous reports (7, 15) had incorrectly suggested that CF centers generally harbor one or more transmissible B. cepacia strains. This statement was contested by other investigators (6, 16, 20). The broad study of CF centers presented in this study, in which several patients at each center were found to harbor the same strain type, is in agreement with previous reports (7, 15, 25) indicating the presence of center-specific predominant B. cepacia strains. RAPD type 2 was the most prevalent CF strain type found (Table 2). The transatlantic spread of this epidemic clone from the United Kingdom where it was originally described (7), to centers in Ontario, Canada, has been documented by others (9, 29), and a possible route of transmission via patient contact at summer camps has been reported (21). We have also shown that this type is widespread among CF patients attending treatment centers outside Ontario, suggesting that this strain type, as others have stated (29), is a hypertransmissible B. cepacia lineage. However, our study of B. cepacia isolates from Canadian treatment centers illustrated that, despite the presence of patients colonized with the epidemic strain (RAPD type 2), other strain types clustered among the patients studied (Fig. 4). These data suggest that the inability of Steinbach et al. (27) to detect evidence of patient-to-patient spread may have been due to the nature of their patient population, which was largely referred from other centers.

The apparent spread of type 6 B. cepacia from one CF patient to other CF patients attending the Vancouver pediatric clinic (Table 1; Fig. 4) further demonstrates the potential risk of patient-to-patient transmission of this organism. One patient alone was chronically colonized with this type 6 strain for 6 years, suggesting low transmissibility by the criteria stated by Steinbach et al. (27). However, in year 7, type 6 was recovered from three other patients, suggesting that this strain type might have spread from one patient to another. Prolonged social contact is an identified risk factor for transmission of B. cepacia (7) and may have accounted for the spread of type 6 B. cepacia in the pediatric clinic. Furthermore, in Vancouver, each prevalent B. cepacia strain type was center specific (Fig. 4), despite the proximity of the pediatric and adult clinics. This suggests that transmission of B. cepacia may have occurred as a result of interaction among patients attending each clinic, although a common source at each center cannot be ruled out.

After blinded typing of the isolates described in this study, the majority of strains were grouped as clonal by RAPD. Five hundred thirty-two isolates were found to belong to 37 distinct fingerprint types, and 95 isolates were unique in their RAPD profile (Tables 1, 2, and 3). CF-associated strain types were generally different from those obtained from other clinical sources and from the environment, and only 6 of the 132 strain types found (types 1, 2, 4, 14, 24, and 39) were recovered from more than one of these sources. The difference between B. cepacia strains recovered from CF patients and those recovered from the environment is in agreement with previous reports (4, 9). We have preliminary data which suggest that B. cepacia strains that are transmissible and infect multiple CF patients (types 1, 2, 4, 6, 13, 17, and 40) harbor a region of the genome, identified by RAPD with primer 272, which is generally absent from isolates colonizing single CF patients and those recovered from other sources (reference 19 and unpublished data).

In conclusion, the RAPD method reported herein is a robust fingerprinting technique which is able to amplify discriminatory polymorphisms from the genome of B. cepacia. The PCR-based technique was suitably versatile to enable a large collection of B. cepacia isolates to be screened without prior knowledge of epidemiology. The method has enabled us to establish the prevalence of various B. cepacia strain types colonizing CF patients treated in Vancouver and monitor the spread of problematic transmissible strain types at other treatment centers. We have also identified two further epidemic B. cepacia strains (types 1 and 4) which infect multiple patients in North America and Europe. These data suggest that other B. cepacia lineages apart from the epidemic type (29) may infect the multiple patients within the global CF community. This method should permit important epidemiological questions to be answered regarding the risk of patient-to-patient spread of B. cepacia in different environments.

ACKNOWLEDGMENTS

We thank Nicole Glenham for excellent technical assistance and Robert Shukin for helpful suggestions concerning DNA extraction and PCR. We acknowledge the Clinic Directors and Microbiologists at Canadian CF treatment centers and Jane Burns, Patricia Ferrieri, John Gowan, Margaret Roy, and Terrence Stull for providing additional B. cepacia isolates. E.M. was supported by a fellowship from the Canadian Cystic Fibrosis Foundation. This work was supported with funds from the Canadian Cystic Fibrosis Foundation.

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