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Species Distribution and Ribotype Diversity of *Burkholderia cepacia* Complex Isolates from French Patients with Cystic Fibrosis

Sylvain Brisse,[1] Christophe Cordevant,[2] Peter Vandamme,[3] Philippe Bidet,[4] Chawki Loukil,[4] Gérard Chabanon,[5] Marc Lange,[2] and Edouard Bingen[4]*

Unité Biodiversité des Bactéries Pathogènes Emergentes (U389 INSERM), Institut Pasteur,[1] Laboratoire d'Études de Génétique Bactérienne dans les Infections de l'Enfant (EA3105), Université Denis Diderot-Paris 7, and Service de Microbiologie, Hopital Robert Debré,[4] Paris, R&D Division, Institut Pasteur de Lille, Lille,[2] and Service de Microbiologie, Hopital Rangueil, Toulouse,[5] France, and Laboratory for Microbiology, Ghent University, Ghent, Belgium[3]

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A total of 153 *Burkholderia cepacia* strains obtained from 153 French patients with cystic fibrosis were identified as *Burkholderia multivorans* (51.6%) or *Burkholderia cenocepacia* (45.1%). Eighty-two genotypes were identified using PvuII and EcoRI ribotyping. *B. multivorans* genotype A (found in 32 French patients) and two other genotypes were also identified among isolates from Austrian, German, Italian, and Canadian patients.

Burkholderia cepacia, originally described as a plant pathogen (10), is now a recognized cause of nosocomial infections (16). *B. cepacia* emerged in the 1980s as an important organism colonizing the lungs of patients with cystic fibrosis (CF) (18). *B. cepacia* acquisition has been associated with accelerated decline of lung function and, in some cases, with respiratory failure and death (16). It is difficult to eradicate pulmonary *B. cepacia* infections (6). Taxonomic studies have shown that the *B. cepacia* complex comprises at least nine closely related species, or genomovars (29). The genomovars are difficult to identify by phenotyping methods (8, 25, 31, 32). Identifying the species and genotype distribution within a given country could have important implications for infection control, clinical outcome, and studies of bacterial virulence and host susceptibility (13). Neither the epidemiology of *B. cepacia* complex nor its genomovar distribution has been studied in French CF patients.

The main purpose of this study was to determine the distribution of *B. cepacia* complex genomovars in French CF centers. As there are diverse strains in the genomovars, we used ribotyping to compare these isolates and sought a possible genomovar-ribotype correlation. Ribotyping has previously proved valuable for discriminating among *B. cepacia* complex isolates (4, 5, 9). We also compared the French isolates with strains from other countries.

A total of 153 *B. cepacia* complex isolates were obtained from 153 patients in 27 French CF centers between 1995 and 2000. These patients received routine care at their CF center, were infected with *B. cepacia* during the study period, and had not been infected previously (26). All the isolates studied were the first *B. cepacia* isolates cultured during this period and were sent to us for genomovar and species identification. Prior samples from these patients were negative for *B. cepacia*. Between 1 and 37 isolates were collected per center.

* Corresponding author. Mailing address: Service de Microbiologie, Observatoire B. cepacia Hôpital Robert Debré, 48 Bd. Sérurier, 75019 Paris, France. Phone: 33 (1) 40 03 23 40. Fax: 33 (1) 40 03 24 50. E-mail: edouard.bingen@rdb.ap-hop-paris.fr.

Isolates were first examined by whole-cell protein electrophoresis (23). Detailed taxonomic studies have demonstrated that this method is an appropriate tool to differentiate members of the *B. cepacia* complex from other *Burkholderia* species and biochemically similar bacteria (12, 14). However, these studies also revealed that strains from some *B. cepacia* complex species are misidentified (12, 13, 30). Therefore, all isolates were further identified using HaeIII and MnlI-*recA* restriction fragment length polymorphism (RFLP) (23).

Ribotyping was performed using a RiboPrinter (Qualicon, Wilmington, Del.) and the restriction enzymes EcoRI and PvuII by standard protocols (9). The PvuII ribotype patterns of the French CF isolates were compared with those of the 178 *B. cepacia* complex strains contained in the Genetic Epidemiology Network for Europe (GENE) database (www.ewi.med.uu.nl/gene), which were isolated from the environment ($n = 32$; 11 from Italy and 21 from the United Kingdom) or from CF patients ($n = 124$; 55 from Italy, 31 from The Netherlands, 33 from Germany, 4 from Canada, and 1 from Austria) or were taken from reference culture collections ($n = 22$). The ribotype patterns of two strains representative of the ET12 clone, identified by multilocus enzyme electrophoresis analysis (19) and shown to cause isolated infections and outbreaks both in Ontario, Canada, and the United Kingdom, and 20 reference strains from culture collections are also present in the GENE database (9).

The whole-cell protein profiles (not shown) and the fact that a *recA* amplicon of the expected size was obtained (23) confirmed that all 153 French isolates belonged to the *B. cepacia* complex. Seventy-nine (51.6%) of the isolates exhibited four different *Burkholderia multivorans recA* RFLP profiles, 69 (45.1%) exhibited five different *Burkholderia cenocepacia recA* RFLP profiles (genomovar III), two (1.3%) exhibited two different *Burkholderia pyrrocinia recA* RFLP profiles (genomovar IX), two (1.3%) exhibited one *Burkholderia stabilis recA* RFLP profile (genomovar IV), and one (0.6%) exhibited one *Burkholderia vietnamiensis recA* RFLP profile (genomovar V).

The 153 French *B. cepacia* isolates were ribotyped separately with PvuII and with EcoRI, yielding 62 and 59 ribotype pat-

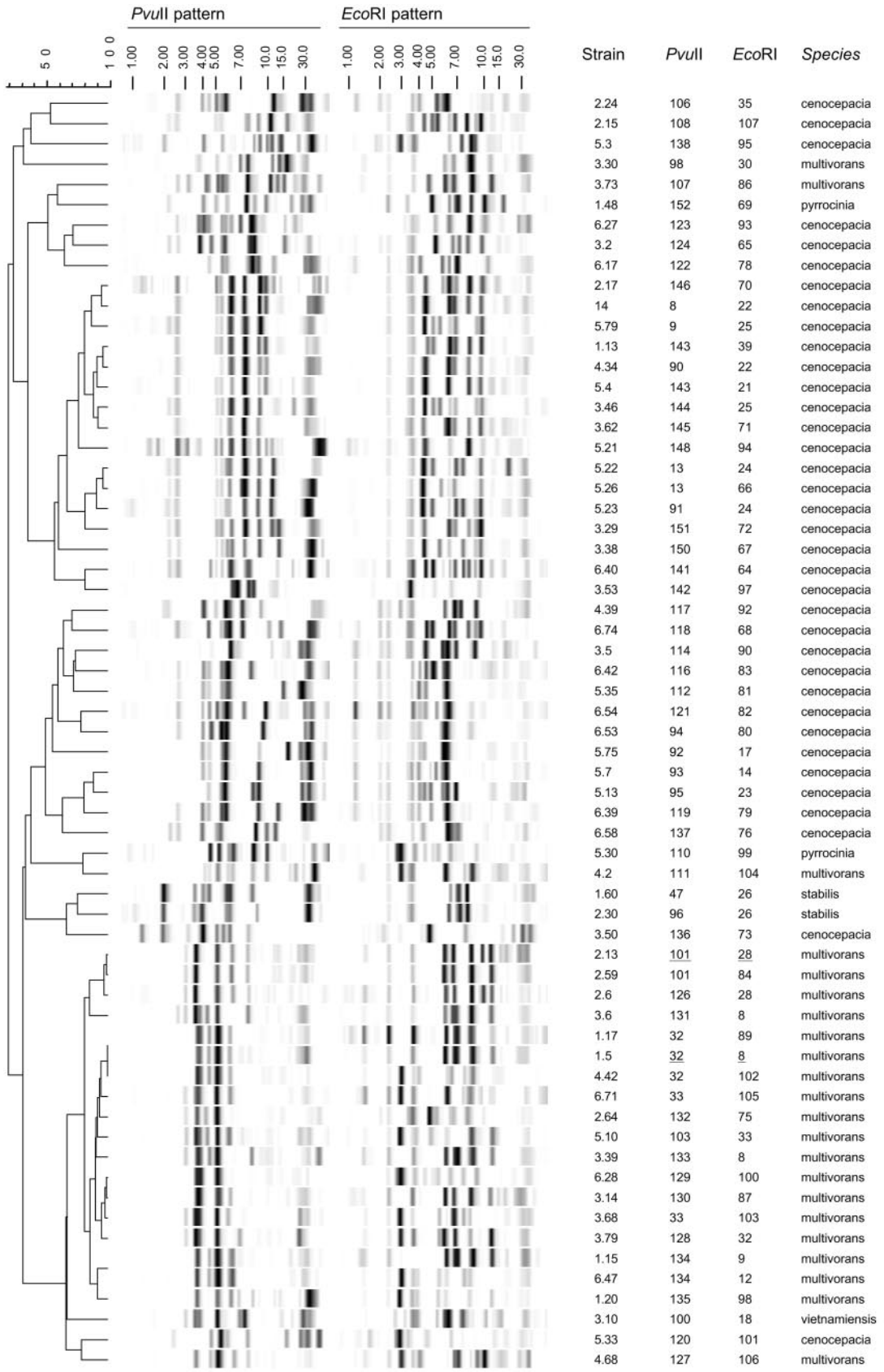

FIG. 1. Overview of distinct PvuII and EcoRI ribotype patterns. The dendrogram was obtained after unweighted pair group method with arithmetic mean clustering of PvuII ribotype patterns using Pearson correlation coefficients (which are transformed into percentage values on the scale). The two major *B. multivorans* genotypes, genotypes A (PvuII-32/EcoRI-8) and B (PvuII-101/EcoRI-28), are underlined. The molecular size scale (in kilobases) is shown above the patterns. The strain codes, PvuII and EcoRI ribotypes, and *Burkholderia* species are shown to the right of the patterns.

terns, respectively (Fig. 1). Combined analysis of the ribotype patterns obtained with the two enzymes yielded 82 genotypes. Two genotypes predominated: genotype A (PvuII-32/EcoRI-8; *B. multivorans*) was identified in 32 patients, and genotype B (PvuII-101/EcoRI-28; *B. multivorans*) was identified in 17 patients. The next most frequent genotype (PvuII-93/EcoRI-14; *B. cenocepacia*) was identified in eight patients.

Each ribotype pattern, obtained with either EcoRI or PvuII, corresponded to a single species. The *B. multivorans* isolates yielded 23 EcoRI and 17 PvuII ribotypes, and the *B. cenocepacia* isolates yielded 44 EcoRI and 39 PvuII ribotypes. The two *B. pyrrocinia* isolates and the *B. vietnamiensis* isolate had distinct ribotypes with each enzyme, while the two *B. stabilis* isolates had the same EcoRI ribotype but distinct PvuII ribotypes. Combined analysis of the ribotype patterns obtained with the two enzymes yielded 26 genotypes for the 79 *B. multivorans* isolates and 51 genotypes for the 69 *B. cenocepacia* isolates. This is the first demonstration that combined use of these two enzymes improves strain discrimination in this setting.

We then compared the PvuII ribotype patterns of the 153 French isolates with those deposited in the GENE database, as ribotype patterns from different riboprinters are reproducible (7). None of the French isolates matched the ribotype patterns of the two ET12 strains, suggesting that the ET12 lineage is absent from French CF centers. Six PvuII ribotypes corresponded to both French CF isolates and GENE database isolates. Two of these ribotypes (PvuII-32 and PvuII-33) corresponded to *B. multivorans*, three (PvuII-8, PvuII-9, and PvuII-13) corresponded to *B. cenocepacia*, and one (PvuII-47) corresponded to *B. stabilis*. We then performed EcoRI ribotyping of the GENE database isolates. All the PvuII ribotypes showed EcoRI ribotype diversity, with the exception of *B. stabilis* PvuII-47. Three combined PvuII-EcoRI genotypes were found among both the French CF isolates and the GENE database isolates. Genotype A (*B. multivorans* PvuII-32/EcoRI-8) was found in 32 French CF patients and one Austrian CF patient. The *B. multivorans* PvuII-33/EcoRI-8 genotype was found in two French patients, four German patients, and one Canadian CF patient. The *B. stabilis* PvuII-47/EcoRI-26 genotype was found in one French patient, two German patients, and one Italian CF patient and in one German water-outlet isolate.

A small number of CF populations in different countries have been characterized in this respect. Our study of the *B. cepacia* complex species distribution in French CF centers shows that *B. multivorans* is the most prevalent (51%), followed by *B. cenocepacia* (45%). Similar results were obtained by others. Heath et al. (17) reported that *B. multivorans* (46%) was slightly more prevalent than *B. cenocepacia* (44%) in 56 American CF patients. Turton et al. (28) reported similar findings (*B. multivorans* [39%], *B. cenocepacia* [29%]) in a study of 111 British CF patients. The high percentage of *B. multivorans* observed in our study suggests interpatient transmission or acquisition from a common source, as only two genotypes were found among 49 CF patients harboring *B. multivorans*. In contrast, Heath et al. (17) found that most of their patients harbored strains with unique genotypes. Similarly, Mahenthiralingam et al. found no evidence of patient-to-patient spread of *B. multivorans* (24). Other studies have found a predominance of *B. cenocepacia*. LiPuma et al. (22), studying 606 patients in the United States, found *B. cenocepacia* in 50% of CF patients and *B. multivorans* in 38% of CF patients. Similar results were found in Portugal, with a *B. cenocepacia* prevalence of 52% (15) and in Australia with a prevalence of 39% (20). In contrast, *B. cenocepacia* is largely predominant in Canada (80%) (27), Italy (72 and 86%) (1, 3), and the United Kingdom (76%) (11). In some studies, other genomovars reach significant percentages, such as genomovar I (36%) and *B. stabilis* (18%) in Portugal (15) or genomovar I in Australia (29%) (20). Differences in species distribution in these studies seem to be due to specificities of the CF subpopulations examined.

We found that isolates belonging to different species always had different ribotypes, confirming the excellent capacity of this approach to distinguish among *B. cepacia* complex species (9). Ribotyping-based species identification of a given isolate can be achieved only if the corresponding ribotype pattern is already present in the database. However, this limitation can be overcome by cluster analysis, as the ribotype patterns obtained for a given species tend to resemble each other and to cluster into a single branch (Fig. 1).

We found that some genotypes, including the frequent genotype A, were present both in French CF patients and in GENE database isolates. These cases possibly correspond to independent acquisition by different patients from natural (environmental) sources in which these strains are highly prevalent (2, 21), but the precise importance of this mode of acquisition remains to be determined (21).

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