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Identification and Population Structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* Genomovar IV)

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The *Burkholderia cepacia* complex currently comprises five genomic species, i.e., *B. cepacia* genomovar I, *B. multivorans* (formerly known as *B. cepacia* genomovar II), *B. cepacia* genomovar III, *B. cepacia* genomovar IV, and *B. vietnamiensis* (also known as *B. cepacia* genomovar V). In the absence of straightforward diagnostic tests for the identification of *B. cepacia* genomovars I, III, and IV, the last two genomic species were not formally classified as novel *Burkholderia* species (genomovar I contains the type strain and therefore retains the name *B. cepacia*). In the present study, we describe differential biochemical tests and a *recA* gene-based PCR assay for the routine identification of strains currently known as *B. cepacia* genomovar IV and propose formal classification of this organism as *Burkholderia stabilis* sp. nov. *B. stabilis* can indeed be differentiated from all other *B. cepacia* complex strains by the absence of beta-galactosidase activity, from strains of *B. cepacia* genomovars I and III and *B. vietnamiensis* by the inability to oxidize sucrose, and from *B. multivorans* by the lack of growth at 42°C. In addition, analysis with the *recA* gene-derived primers BCRG41 (5′-ACCGGCGAGCAG GCGCTT-3′) and BCRG42 (5′-ACGCCATCGGGCATGGCA-3′) specifically allows the detection of *B. stabilis* strains in a conventional PCR assay. Examination of a set of 21 *B. stabilis* strains by means of random amplified polymorphic DNA analysis and pulsed-field gel electrophoresis typing suggested that the genome of this organism is highly conserved, which is in sharp contrast to the generally accepted genomic diversity, variability, and plasticity among *B. cepacia* strains.

Accurate species-level identification of *Burkholderia* strains is often a tedious process. The discovery of a variety of novel species, new taxonomic insights, and the peculiar genomic characteristics of these organisms present diagnostic laboratories with a manifold of problems. Driving forces behind this rapid evolution are the biotechnological interest in biocontrol and bioremediation applications of *Burkholderia*-like organisms, their role as plant pathogens, and, not the least, their role as significant pathogens for particular patient groups such as cystic fibrosis patients (7). A study of the taxonomy of *Burkholderia cepacia*-like organisms revealed the complex nature of this organism (23). The name *B. cepacia* complex was proposed to comprise a cluster of five closely related species, originally referred to as *B. cepacia* genomovars I through V (23) (the term genomovar was introduced to denote phenotypically similar but genotypically distinct groups of strains [22]). Apart from genomovar I, which contains the type strain of *B. cepacia*, two of these genomovars have been formally named (*Burkholderia multivorans* and *Burkholderia vietnamiensis* for strains previously known as *B. cepacia* genomovars II and V, respectively); the others await assignment of a binomial species name pending the availability of distinguishing phenotypic identification criteria (23).

We recently reported on the application of a genomic fingerprinting technique, amplified fragment length polymorphism (AFLP) analysis, for the differentiation of members of the *B. cepacia* complex (5). In that study, we demonstrated that several strains classified as *B. cepacia* genomovar I by means of whole-cell protein electrophoresis (23) belonged, in fact, to *B. cepacia* genomovar IV. While examining additional putative *B. cepacia* strains, we repeatedly identified genomovar IV strains by AFLP analysis but not by whole-cell protein electrophoresis. In this report we describe effective procedures for the identification of *B. cepacia* genomovar IV strains and propose the name *Burkholderia stabilis* sp. nov. to accommodate *B. cepacia* genomovar IV strains.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Reference strains of *B. cepacia* genomovars I (13 strains) and III (3 strains) and of *B. multivorans* (3 strains), *B. vietnamiensis* (3 strains), and *Burkholderia gladioli* (3 strains), have been described in previous reports (5, 23). Three *Burkholderia pseudomallei* reference strains were obtained from the CCUG Culture Collection (Culture Collection of the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden). All 21 *B. stabilis* strains and their sources are listed in Table 1.

Bacteriological purity was checked by plating and examining living cells by phase-contrast microscopy and Gram staining of cells. Strains were grown on nutrient agar (Oxoid CM3 agar) supplemented with 0.04% (wt/vol) KH_2PO_4 and 0.24% (wt/vol) $Na_2HPO_4 \cdot 12H_2O$ (pH 6.8) and incubated aerobically at 28°C unless indicated otherwise.

AFLP fingerprinting. DNA was isolated and purified as described by Pitcher et al. (15). AFLP analysis was performed as described by Coenye et al. (5). Preparation of template DNA for PCR analysis, preselective and selective PCR amplification with 6-carboxyfluorescein-labeled primers, separation of amplified fragments on an ABI Prism 377 DNA Sequencer, and data capture and analysis with GeneScan (version 2.1; Perkin-Elmer Applied Biosystems) and GelCompar (version 4.2; Applied Maths, Kortrijk, Belgium) software were performed as described previously (5).

PAGE of whole-cell proteins. All reference strains and *B. stabilis* strains were included in the sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis. After an incubation period of 48 h, whole-cell protein extracts

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TABLE 1. *B. stabilis* strains studied

Strain	Other strain no.[a]	Source (country, yr of isolation)
LMG 6997	CCUG 3461B	Ear (Sweden, 1974)
LMG 7000	CCUG 13348	Blood (Sweden, 1983)
LMG 13017		Blood culture (Belgium, 1988)
LMG 14086		Respirator (United Kingdom, 1970)
LMG 14291		Cystic fibrosis patient (Belgium, 1993)
LMG 14294^T	NCTC 13011	Cystic fibrosis patient (Belgium, 1993)
LMG 14295		Cystic fibrosis patient (Belgium, 1993)
LMG 14940		Cystic fibrosis patient (Belgium, 1994)
LMG 15949		Cystic fibrosis patient (Belgium, 1993)
LMG 15950		Water bath, cystic fibrosis ward (Belgium, 1994)
LMG 15951		Spirometer (Belgium, 1994)
LMG 18138	E20	Cystic fibrosis patient (Belgium, 1995)
R-3338	M71-40	Cystic fibrosis patient (Germany, 1997)
R-6617	J687	Human, non-cystic fibrosis patient (France)
R-6618	J762	Urine (United States)
LMG 18888	HK 268a	Human blood (Belgium, 1995)
R-136	J1750	Cystic fibrosis patient (United States before 1989)
R-4059	H107	Cystic fibrosis patient (Germany, 1993)
R-732	C6061	Cystic fibrosis patient (Canada, 1994)
R-737	CEP059	Respiratory tract, non-cystic fibrosis patient (Canada)
R-741	CEP194, J668	Human, non-cystic fibrosis patient (Switzerland)

[a] CCUG, Culture Collection of the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; LMG, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Ghent, Belgium; NCTC, National Collection of Type Cultures, London, United Kingdom.

were prepared and SDS-PAGE was performed as described before (16). The densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis were performed with the GelCompar software package (version 4.2; Applied Maths). Similarity levels between the patterns were calculated by using the Pearson product moment correlation coefficient and are expressed as percent similarity for convenience. Data for the reference strains of *B. cepacia* genomovars I, II (*B. multivorans*), III, and V (*B. vietnamiensis*) and for *B. gladioli* were generated in a previous study (23).

Conventional biochemical tests. The following tests were performed with all 21 *B. stabilis* strains listed in Table 1 as described previously (8). Briefly, pure cultures were stored at −70°C in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 8% dimethyl sulfoxide. Frozen isolates were subcultured onto Columbia agar containing 5% sheep blood (PML Microbiologicals, Richmond, British Columbia, Canada) before testing. The primary identification system used was the API Rapid NE system (Biomerieux Vitek Inc., Hazelwood, Mo.) supplemented with glucose, maltose, lactose, xylose, sucrose, and adonitol oxidation-fermentation (OF) sugars (10) and an adaptation of Moeller lysine and ornithine decarboxylases (Difco) (the 2-ml test volume was overlaid with 0.5 ml of oil and a heavy, visible inoculum was used; a tube without amino acid was included as a negative control). The ability to grow on BCSA agar (8), indicating resistance to gentamicin and polymyxin, was tested. Incubation was at 35°C in ambient air; tubes with OF sugars were incubated for up to 7 days, and the tubes used for the other tests were incubated for 2 days. Any organism that was *P*-nitrophenyl-β-D-glucoside (PNPG) negative on the API strip was tested for the presence of beta-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside [ONPG] test) (1). In addition, growth on tryptic soy agar (Becton Dickinson and Co., Cockeysville, Md.) at 35 and 42°C was observed for appearance and pigmentation.

In addition, a range of 68 conventional biochemical tests was performed by previously described methods (9) with eight *B. stabilis* strains (strains LMG 6997, LMG 7000, LMG 14086, LMG 14291, LMG 14294, LMG 14295, LMG 14940, and LMG 15949) as reported in a previous study (23). Except for the ONPG test, data for *B. cepacia* genomovars I and III, *B. multivorans*, and *B. vietnamiensis* were taken from a previous study (23). Unpublished data from D. Henry showed that strains from these reference species all exhibit beta-galactosidase activity.

PCR assay. Assays for the identification of *B. cepacia* complex genomovars were based on the gene encoding RecA and are described in detail in a separate report (E. Mahenthiralingam, J. M. Bischof, S. K. Byrne, C. Radomski, J. E. Davies, Y. Av-Gay, and P. Vandamme, submitted for publication). The nucleotide sequences of the entire *recA* genes from *B. stabilis* LMG 7000 and LMG 14291 were determined. These sequences were aligned by using the CLUSTAL W algorithm (21) with the sequences of *recA* genes from strains representative of *B. cepacia* genomovars I and III, *B. multivorans*, and *B. vietnamiensis*. This alignment facilitated the design of PCR primers BCRG41 (5′-ACCGGCGAG CAGGCGCTT-3′) and BCRG42 (5′-ACGCCATCGGGCATGGCA-3′) which were specific to the *B. stabilis recA* sequence and which were mismatched at the 3′ base with *recA* sequences from all remaining genomovars. Under standard PCR conditions (14) at an annealing temperature of 64°C, a product of 647 bp was specifically amplified from the *B. stabilis* strains. Strains of the other genomovars failed to produce a PCR product under these conditions (Mahenthiralingam et al., submitted).

Genetic typing. Genetic typing of *B. stabilis* strains was performed both by random amplified polymorphic DNA (RAPD) analysis as described previously (13) and by pulsed-field gel electrophoresis (PFGE) fingerprinting. The PFGE method was adapted from that of Cheng and Lessie (3) and was performed as follows. After overnight growth in 5 ml of Luria-Bertani broth (19), the bacteria were harvested by centrifugation and were resuspended to an optical density at 620 nm of between 0.8 and 0.9 in SE buffer (75 mM NaCl, 25 mM EDTA [pH 7.4]). The suspension was warmed to 45°C for 5 min and was mixed with an equal volume of molten 2% low-melting-point agarose (Type 7; Sigma-Aldrich Canada, Oakville, Ontario, Canada) that was kept at the same temperature, and the mixture was poured into 70-μl disposable plug molds (Bio-Rad, Mississauga, Ontario, Canada). The plugs were briefly chilled to 4°C, and then three to five plugs were placed in 10 ml of PEN buffer (0.5 M EDTA [pH 9.6], 1% *N*-lauroyl sarcosine) containing 1 mg of pronase (Boehringer Mannheim, Laval, Quebec, Canada) per ml held within a 15-ml sterile tube. After 24 h of incubation with gentle rocking at 37°C, the plugs were washed with five volume changes (one per hour) of TE buffer (Tris-EDTA) (19). Slices (approximately 2 mm) were then cut from the plugs and were incubated overnight with 10 U of *Spe*I in a 150-μl digestion mixture at 37°C. The macrorestricted DNA was separated in 1.2% agarose gels made with 0.5× TBE buffer (Tris-borate-EDTA) (19) at 5 V/cm for 44 h, with pulse switch times ramped from 20 to 60 s according to the manufacturer's standard guidelines (CHEF-DR II apparatus; Bio-Rad). Bacteriophage lambda concatemers were included as size standards (Bio-Rad).

The normalization of the banding patterns that were obtained and the numerical analysis with the Pearson product moment correlation coefficient were performed with the GelCompar software package (version 4.2; Applied Maths).

16S rDNA sequence analysis. Preparation of DNA, amplification of part of the rRNA gene (rDNA) operon comprising the nearly complete 16S DNA, and sequence analysis and assembly were performed as described before (4, 24).

Nucleotide sequence accession numbers. The GenBank nucleotide sequence accession number for the 16S rDNA sequence of strain LMG 14294 is AF148554. The nucleotide sequence accession numbers for the *recA* genes of *B. stabilis* strains LMG 7000 and LMG 14291 are AF143789 and AF143790, respectively.

RESULTS

AFLP fingerprinting. The intergel reproducibility level was higher than 93%; the intragel correlation between patterns was higher than 95% (data not shown). Identification of strains was achieved by numerical analysis of the AFLP patterns of novel isolates with those of a well-characterized set of reference strains described by Coenye et al. (5) (data not shown).

PAGE of whole-cell proteins. Duplicate protein extracts were prepared to check the reproducibility of the growth con-

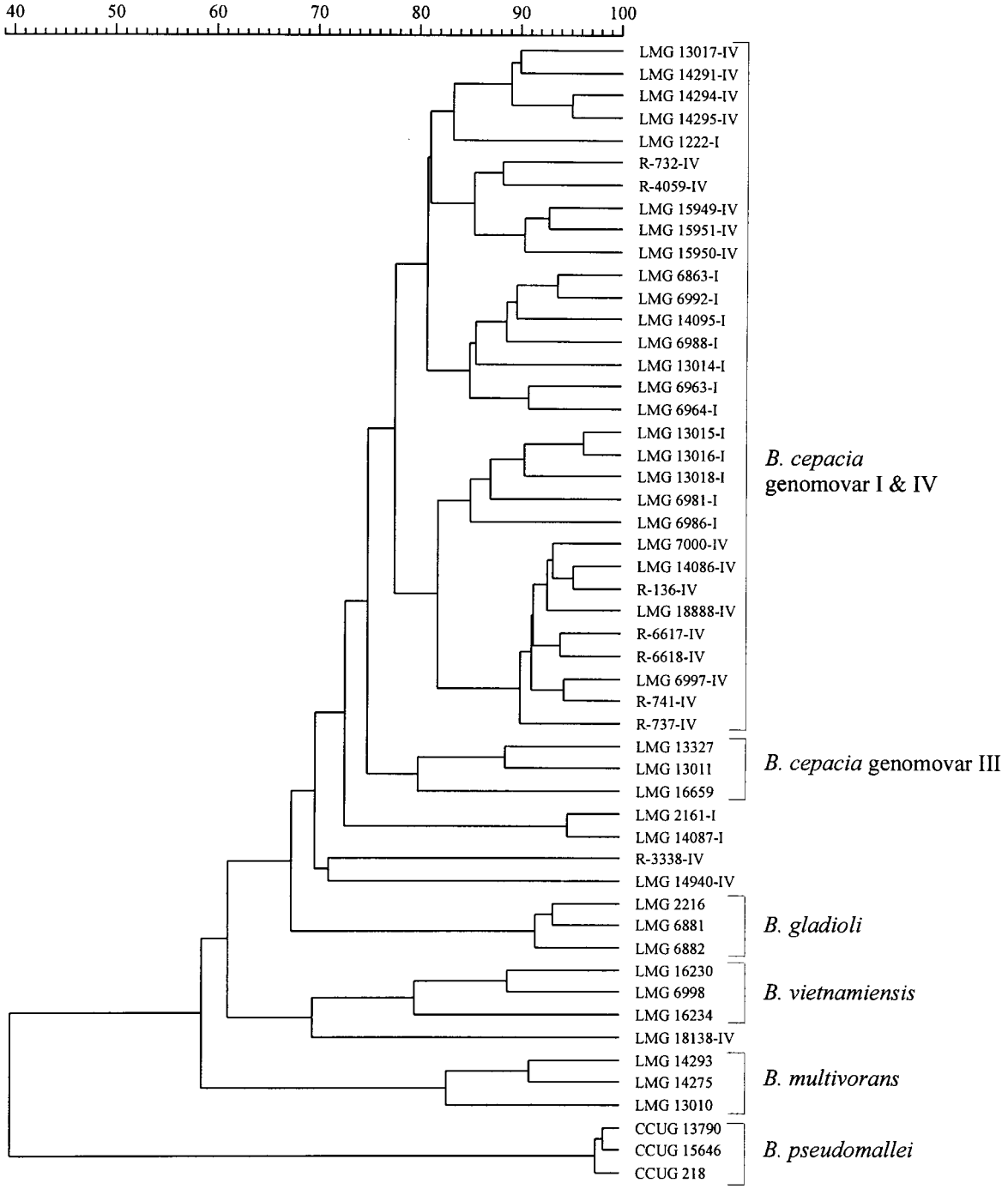

FIG. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients between the protein patterns of all strains studied. Strain numbers with the suffix I or IV refer to *B. cepacia* genomovar I or *B. stabilis* strains, respectively.

ditions and the preparation of the extracts. The correlation level between duplicate protein patterns was more than 93% (data not shown).

Figure 1 shows the result of the numerical analysis of the whole-cell protein patterns of reference strains of the various *B. cepacia* genomovars and of *B. gladioli* and *B. pseudomallei*. Strains of the last two species and of *B. multivorans*, *B. vietnamiensis*, and *B. cepacia* genomovar III each form well-delineated clusters. However, the majority of *B. cepacia* genomovar I and *B. stabilis* strains form a single heterogeneous cluster above a similarity level of 77.2%. In addition, some *B. cepacia* genomovar I strains (strains LMG 2161 and LMG 14087) and *B. stabilis* strains (strains LMG 14940, R-3338, and LMG 18138) occupy distinct positions in the dendrogram.

Conventional biochemical tests. The results of those tests that were performed with all 21 *B. stabilis* strains are as follows. All 21 *B. stabilis* strains were negative for nitrate reduction, ONPG, esculin hydrolysis, growth at 42°C, and sucrose oxida-

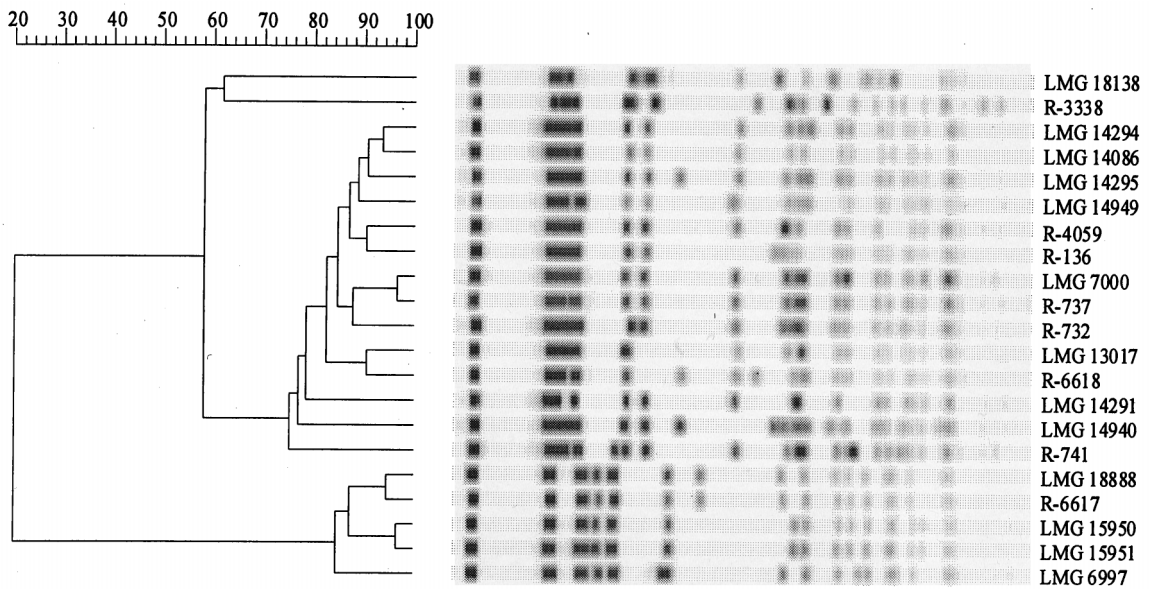

FIG. 2. Computer-generated *Spe*I-PFGE profiles of all of the *B. stabilis* strains and the corresponding numerical analysis of the banding patterns.

tion. All 21 strains were positive for acidification of glucose, lactose, maltose, xylose, and adonitol, liquefaction of gelatin, and lysine and ornithine decarboxylation. All 21 strains demonstrated a weakly positive oxidase reaction. The API Rapid NE system gave profile numbers of 0046577 (for five strains that did not liquefy gelatin) or 0056577 (for the remaining strains that liquefied gelatin). Tube gelatin tests gave positive results for all except one (strain R-3338) of the strains that were negative with the API Rapid NE system. In the API identification database (API 20 NE, version 6), the identification result for strains with the profile 0056577 corresponded to a "good identification" score for *B. cepacia*, followed by *Pseudomonas fluorescens* as the second choice; the note "possibility of *B. gladioli*" was given, too. The same identification results were obtained for strains with profile 0046577, but with "low discrimination" instead of "good identification." All strains grew on BCSA agar, indicating resistance to gentamicin and polymyxin. No production of pigments was observed.

The results of the 68 tests performed with the restricted set of eight strains are listed in the description of the *B. stabilis* strains given below.

PCR assay. All of the *B. stabilis* strains tested reacted positively with the *B. stabilis recA*-specific primers. None of the strains representing other genomovars or *Burkholderia* species reacted positively. The PCR product of the correct size from strain LMG 7000 was confirmed to encode the *recA* gene by direct nucleotide sequence analysis (data not shown).

Genetic strain typing. Reproducible and discriminatory genetic fingerprints were obtained for *B. stabilis* strains examined by RAPD analysis (data not shown) and PFGE. Figure 2 is a computer-based reproduction of the *Spe*I-PFGE profiles of all *B. stabilis* strains and the results of the corresponding numerical analysis of the banding patterns. Two main clusters with very similar banding patterns and two strains with unique profiles (strains LMG 18138 and R-3338) can be distinguished. RAPD fingerprinting identified the same strain clusters (data not shown).

DISCUSSION

The *B. cepacia* complex currently comprises five genomic species originally referred to as *B. cepacia* genomovars I through V, respectively (23). One of these genomic species (genomovar V) was identified as *B. vietnamiensis*, a nitrogen-fixing bacterium originally isolated from the rice rhizosphere (6). Of the remaining four genomic species, only genomovar II could readily be differentiated from the others by the absence of sucrose utilization and variable lysine decarboxylase activity, which were typically present in other *B. cepacia* complex strains (23); this genomovar was named *B. multivorans*. In the absence of straightforward differential tests, the remaining genomic species were referred to as *B. cepacia* genomovars I, III, and IV (23).

Identification of *B. stabilis* strains. Previous AFLP analyses and subsequent DNA-DNA hybridization experiments revealed that some of the strains used when searching for differential tests for the separation of *B. cepacia* genomovar I, III, and IV strains were misidentified, as some *B. cepacia* genomovar I strains were shown to be genomovar IV (5). Continued application of AFLP analysis as a first-line identification approach in a research laboratory identified a set of 21 strains as *B. cepacia* genomovar IV. Subsequent analyses of these strains by classical biochemical tests revealed that genomovar IV strains can be differentiated from other *B. cepacia* complex strains by the absence of the beta-galactosidase activity necessary for the breakdown of ONPG. In addition, the inability to oxidize sucrose separates them from strains of genomovars I and III and *B. vietnamiensis* but not from *B. multivorans*. The lack of growth at 42°C further separates *B. cepacia* genomovar IV strains from *B. multivorans* strains.

In addition, PCR technology offers alternative possibilities for the detection of organisms where species-level identification is particularly difficult. Bauernfeind et al. (2) recently described an assay that facilitated discrimination of *B. multivorans* and *B. vietnamiensis* strains from *B. cepacia* genomovar I, III, and IV strains and that was based on specific regions in the rRNA operon. This assay, however, did not allow differ-

TABLE 2. Fatty acid compositions of the strains studied[a]

Fatty acid	Fatty acid composition (%)[b]					
	B. cepacia genomovar I (14 strains)	*B. multivorans* (14 strains)	*B. cepacia* genomovar III (48 strains)	*B. stabilis* (11 strains)	*B. vietnamiensis* (4 strains)	*B. gladioli* (4 strains)
14:0	3.7 ± 1.6	4.8 ± 0.6	4.3 ± 0.7	4.4 ± 0.5	3.8 ± 0.6	4.9 ± 0.2
16:1 ω7c	4.9 ± 2.8	6.5 ± 2.5	3.3 ± 1.6	3.3 ± 1.2	9.8 ± 2.5	4.4 ± 2.3
16:0	26.8 ± 2.8	28.9 ± 3.5	26.5 ± 3.3	25.6 ± 4.3	19.5 ± 2.5	29.0 ± 1.1
17:0 cyclo	17.9 ± 3.6	18.2 ± 4.9	22.3 ± 4.3	17.8 ± 3.3	14.0 ± 4.9	17.2 ± 1.9
16:1 2OH	Tr (11)[c]	1.3 ± 0.8 (12)	1.5 ± 1.0 (42)	Tr (8)	1.3 ± 0.2	1.5 ± 0.2
16:0 2OH	3.3 ± 0.9	2.8 ± 1.0	3.0 ± 0.9	4.5 ± 1.5	2.8 ± 0.4	3.2 ± 0.3
16:0 3OH	6.6 ± 0.9	6.8 ± 0.5	6.1 ± 1.2	5.7 ± 0.9	6.4 ± 0.7	7.1 ± 1.0
18:1	12.4 ± 4.0	11.5 ± 4.8	9.4 ± 4.9	10.6 ± 1.6	19.7 ± 5.5	9.8 ± 3.9
18:0	Tr (13)	Tr (5)	2.3 ± 2.2	Tr (8)	5.2 ± 3.4	Tr
19:0 cyclo ω8c	12.5 ± 4.7	9.7 ± 3.3	12.3 ± 4.3	15.3 ± 4.2	5.8 ± 2.5	10.0 ± 2.7
18:1 2OH	3.4 ± 0.8	1.3 ± 0.7 (12)	1.6 ± 1.4	2.4 ± 0.6	3.4 ± 0.5	4.1 ± 1.3
14:0 3OH	6.2 ± 1.6	6.4 ± 1.1	6.1 ± 1.1	5.4 ± 0.8	6.3 ± 0.9	6.8 ± 0.9

[a] Data were generated and taken from a previous study (23), but new average profiles were calculated in light of the corrected classification of several *B. stabilis* strains (see text).

[b] Those fatty acids for which the average amount for all taxa was less than 1% are not given. Therefore, the percentages for each group do not total 100%. Tr, trace amount (less than 1%).

[c] The numbers in parentheses refer to the numbers of strains containing this fatty acid.

entiation of the last three genomovars. Mahenthiralingam et al. (submitted) described a different approach based on polymorphisms of the *recA* genes. Their *B. cepacia* genomovar IV-specific primers proved to be highly specific and sensitive and facilitated identification of all the *B. cepacia* genomovar IV strains examined in the present study.

The results of the present study corroborated the failure of one-dimensional whole-cell protein electrophoresis to distinguish between *B. cepacia* genomovar I and IV strains (Fig. 1). We previously reported on the failure of whole-cell fatty acid analysis to distinguish between members of the *B. cepacia* complex (23). Reevaluation of these same previously published data in light of the corrected classification of several *B. cepacia* genomovar IV strains corroborated our previous findings, as the different *B. cepacia* genomovars and *B. gladioli* remained virtually indistinguishable (Table 2).

By use of the procedures for the identification of *B. cepacia* genomovar IV listed above, all required criteria are available to propose an official binomial species name for this organism (22). We therefore propose the name *B. stabilis* to accommodate the former *B. cepacia* genomovar IV strains.

Population structure of *B. stabilis*. A variety of typing studies have been performed with *B. cepacia*-like organisms (13, 17, 20, 25, 26). One of the general conclusions of such studies was that the extent of genetic diversity within this group of bacteria is extremely high. In the present study, we examined 21 *B. stabilis* strains by means of RAPD analysis and PFGE, both of which are highly discriminatory fingerprinting methods (3, 13). These strains were isolated over a period of nearly 30 years (1970 until 1997) from sputum samples of cystic fibrosis patients (10 strains), different infections in non-cystic fibrosis patients (8 strains), and a number of sources of the hospital environment (3 strains) (Table 1). The geographic origins of the strains were very diverse: Belgium (10 strains), Canada (2 strains), France (1 strain), Germany (2 strains), Sweden (2 strains), Switzerland (1 strain), the United Kingdom (1 strain), and the United States (2 strains) (Table 1). Three of these isolates (isolates LMG 14294, LMG 14295, and LMG 15949) were cultured from sputum specimens of one cystic fibrosis patient over a 6-month period, while strain LMG 14940 was obtained from another cystic fibrosis patient attending the same clinic. Furthermore, there is an epidemiological link between strains LMG 15950 and LMG 15951, as both were isolated in 1994 from equipment of different wards of a single Belgian hospital. There was, however, no apparent relationship between any of the other isolates. Yet, the genomic variability among these strains was remarkably restricted. Numerical analysis of the PFGE fingerprinting patterns of these strains revealed only two main clusters of strains (Fig. 2); two additional strains, LMG 18138 and R-3338, were characterized by unique PFGE fingerprints. Although there were obvious differences among some of the strains within each of the clusters, the overall profiles were remarkably conserved. This genomic stability is in sharp contrast to the reported genomic diversity among *B. cepacia* strains in general and to the generally accepted genomic plasticity and variability of these organisms (11, 12, 18, 26). These data indicate that findings that are valid for some *B. cepacia*-like strains should not be extrapolated to the entire *B. cepacia* complex or even to the related species. They also indicate that interpretation of the similarity of DNA profiles for epidemiological investigations should be done with extreme caution.

Description of *B. stabilis* sp. nov. *Burkholderia stabilis* (sta′bi.lis. L. adj. *stabilis*, stable, permanent, referring to the relative genomic stability of this *B. cepacia* genomovar) cells are motile rods that are 1.0 to 2.0 μm long and 0.6 to 0.9 μm wide (the description is based on data obtained in the present study for all 21 strains and on data reported previously [23] for strains LMG 6997, LMG 7000, LMG 14086, LMG 14291, LMG 14294, LMG 14295, LMG 14940, and LMG 15949). Growth is observed at room temperature and at 37°C but not at 42 or 5°C. So far, no pigmented strains have been detected and no melanin-like pigment is produced on tyrosine agar. There is growth on MacConkey agar and Simmons citrate agar. There is oxidation in OF medium, an alkaline reaction on Christensen's citrate agar, and growth in the presence of cetrimide. There is no reduction of 0.4% selenite. Tolerance to KCN is strain dependent (two of eight strains tested showed weak growth; all others were negative). There is no fluorescence on King's B medium. Tyrosine, Tween 20, and Tween 80 are hydrolyzed. Catalase, oxidase, and lecithinase activities are present; urease and beta-galactosidase (ONPG test) activities are absent. Nitrate reduction is strain dependent when it is determined as described by Holmes et al. (9) (three of eight

strains tested reduced nitrate; all others did not) but is uniformly negative with the API strip; nitrite is not reduced. There is no indole, hydrogen sulfide, or 3-ketolactose production, no hydrolysis of esculin or starch, and no DNase activity. Liquefaction of gelatin and hydrolysis of casein are strain dependent (16 of 21 strains tested liquefied gelatin by the API assay; all but 1 of the strains liquefied gelatin when liquefaction was determined by a tube test [8]; 2 of 8 strains tested liquefied gelatin when liquefaction was determined by a plate test [9]; 7 of 8 strains tested hydrolyzed casein). Arginine dihydrolase and arginine desimidase activities are absent. Lysine and ornithine decarboxylase activities are absent when the activities were tested for as described by Barrow and Feltham (1) but were present when the activities were tested for as described in the present study. There is no oxidation of gluconate and no production of phenylpyruvic acid, and all strains utilize malonate. Poly-beta-hydroxybutyrate is utilized and is present as inclusion granules. Acid but no gas is produced from glucose-peptone-water sugar. Acid is produced from 10% (wt/vol) glucose, from 10% (wt/vol) lactose, and from the following sugars in ammonium salt medium: glucose, adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, lactose, maltose, mannitol, sorbitol, trehalose, xylose, and fructose. Acid is not produced (or is only weakly produced) from raffinose, rhamnose, ethanol, or sucrose. Acid production from salicin is strain dependent (three of eight strains tested produced acid from salicin).

The DNA base ratio is 68 to 69 mol%. Major fatty acid components are 14:0 (about 4.4%), 14:0 3OH (about 5.4%), 16:1 ω7c (about 3.3%), 16:0 (about 25.6%), 17:0 cyclo (about 17.8%), 16:0 3OH (about 5.7%), 18:1 (about 10.6%), and 19:0 cyclo ω8c (about 15.3%).

B. stabilis strains have been isolated from the sputum of cystic fibrosis patients, from blood, ear, and respiratory tract infections in non-cystic fibrosis patients, and from the hospital environment. The full range of pathogenicity is incompletely understood. The type strain is LMG 14294, which was isolated in 1993 in Leuven, Belgium, from the sputum of a cystic fibrosis patient. Its DNA base ratio is 68 mol%, and its phenotypic characteristics are as described above for the species. The database accession number of its 16S rRNA gene sequence is AF148554.

The type strain and several additional strains are available from the Belgian Co-ordinated Collections of micro-organisms/Laboratorium Microbiologie Gent Culture Collection and National Collection of Type Cultures culture collections.

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