

Occurrence of Multiple Genomovars of *Burkholderia cepacia* in Cystic Fibrosis Patients and Proposal of *Burkholderia multivorans* sp. nov.

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We performed an integrated genotypic and phenotypic analysis of 128 strains of the genera *Burkholderia*, *Ralstonia*, and *Pseudomonas* in order to study the taxonomic structure of *Burkholderia cepacia* and its relationships with other *Burkholderia* species. Our data show that presumed *B. cepacia* strains isolated from cystic fibrosis patients belong to at least five distinct genomic species, one of which was identified as *Burkholderia vietnamiensis*. This group of five phenotypically similar species is referred to as the *B. cepacia* complex. The name *Burkholderia multivorans* is proposed for one of these genomic species, which was formerly referred to as *B. cepacia* genomovar II; the remaining *B. cepacia* groups are referred to as genomovars I, III, and IV, pending additional differential phenotypic tests. The role and pathogenic potential of each of these taxa, particularly in view of the potentially fatal infections in cystic fibrosis patients, need further evaluation. The data presented also demonstrate that *Pseudomonas glathei* and *Pseudomonas pyrocinia* should be reclassified as *Burkholderia* species.

The taxonomy and the description of the genus *Pseudomonas* have been changing for more than a century (16). The discovery of five major phylogenetic lineages has played a major role (23) and was at the origin of the description of several new genera, the authentic pseudomonads being confined to those species belonging to the same lineage as *Pseudomonas aeruginosa*, the type species (9, 23). Yabuuchi et al. (43) reclassified a number of *Pseudomonas* species belonging to rRNA group II (21) as *Burkholderia* species. However, these authors examined only some of the species belonging to rRNA group II, and their conclusions were based on a limited set of strains. As a consequence, several additional *Pseudomonas* species had to be reclassified as *Burkholderia* species in subsequent studies (10, 35, 46) and the genus *Ralstonia* was created to accommodate the generically misclassified species *Burkholderia solanacearum* and *Burkholderia pickettii* (10, 44). At present, the genus *Burkholderia* comprises 11 species: *Burkholderia andropogonis*, *Burkholderia caryophylli*, *Burkholderia cepacia* (the type species), *Burkholderia cocovenenans*, *Burkholderia gladioli*, *Burkholderia glumae*, *Burkholderia mallei*, *Burkholderia plantarii*, *Burkholderia pseudomallei*, *Burkholderia vandii*, and *Burkholderia vietnamiensis* (10).

During the last decade, *B. cepacia*, traditionally a plant pathogen occurring in diverse ecological niches, has caused great concern among cystic fibrosis patients and their caregivers. The narrow spectrum of bacteria causing severe pulmonary exacerbations in cystic fibrosis patients was traditionally confined to *Staphylococcus aureus*, *Haemophilus influenzae*, and *P. aeruginosa* (12). Since the early 1980s, increasing numbers of *B. cepacia* infections have been reported in several

countries (1, 12). Infection or colonization by *B. cepacia* strains led to different outcomes in different patients. A major cause for concern was that about 20% of patients succumb to *B. cepacia* syndrome, a necrotizing pneumonia which culminates in rapid and fatal clinical deterioration (12, 15). Other patients are chronically colonized without, or with only a slow, deterioration of lung function (11, 26, 28). In addition, several cases of *B. gladioli* infections or infections caused by organisms with characteristics seemingly intermediate between *B. gladioli* and *B. cepacia* were reported (5, 28).

The term genomovar was recently introduced to denote phenotypically similar but genotypically distinct groups of strains that were previously referred to by a variety of different terms including genomic species, genomic groups, genospecies, or genomospecies (36). These genomovars share a low level of DNA hybridization and therefore represent distinct species for which an official binomial name is not proposed pending the availability of differential diagnostic tests.

The present study was initiated by the receipt, at about the same time, of several strains taken from cystic fibrosis patients from two different centers in the English Midlands for computer-assisted identification at the National Collection of Type Cultures (NCTC). The strains were difficult to identify because they were phenotypically heterogeneous, with some being non-saccharolytic while others were saccharolytic and closely resembled *B. cepacia*. Nevertheless, the isolates had virtually identical whole-cell protein patterns as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). As concern regarding *B. cepacia* increased (1) and laboratory identification improved, such strains were recognized from other parts of the United Kingdom and from Belgium during a national surveillance study (26). In addition, several studies revealed considerable heterogeneity among strains tentatively classified as *B. cepacia* (2, 3, 10, 28, 33, 34,

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45). These initial observations prompted the fuller polyphasic study described herein.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All strains studied and their sources are listed in Table 1. Thirty-nine reference strains were obtained from international culture collections. In addition, 89 recent isolates identified as *B. cepacia* by means of biochemical tests, cellular fatty acid analysis, or both were included. All reference strains and a selection of the field isolates examined (see below) are available from the Belgian Coordinated Collections of Microorganisms/Laboratorium Microbiologie Gent (BCCM/LMG) and NCTC culture collections.

Bacteriological purity was checked by plating, gram staining, and examining living cells by phase contrast microscopy. Strains were grown on nutrient agar containing 0.1% (wt/vol) beef extract, 0.2% yeast extract, 0.5% (wt/vol) NaCl, 0.5% (wt/vol) peptone, 0.04% (wt/vol) KH_2PO_4 , 0.24% (wt/vol) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, and 2.0% (wt/vol) agar (pH 6.8) and incubated aerobically at 28°C unless indicated otherwise.

PAGE of whole-cell proteins. After an incubation period of 48 h, whole-cell protein extracts were prepared and SDS-PAGE was performed as described before (25). The densitometric analysis, normalization and interpolation of the protein profiles, and numerical analysis were performed by using the GelCompar software package, version 4.0 (Applied Maths, Kortrijk, Belgium).

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described before (39). DNA was extracted from representative strains of all clusters or subclusters containing presumed *B. cepacia* strains: i, v, vi, vii, viii, x, xi, xii, and xiii (see below). DNA was also prepared from *B. cepacia* LMG 14939, which occupied a separate position in the dendrogram, and from the *B. coveneniensis*, *B. gladioli*, *P. glathei*, and *P. pyrrocinia* type strains.

DNA base compositions. All of the mean moles percent guanine plus cytosine values were determined by thermal denaturation and calculated as described by De Ley (7).

DNA-rRNA hybridization experiments. In vivo ^3H -labelled 23S rRNA from *B. cepacia* LMG 1222^T was available from previous studies (10). Purification of rRNA fractions, fixation of single-stranded DNA on membrane filters, chemical determination of the amount of DNA on the filter, saturation hybridization, ribonuclease treatment, and thermostability measurement of the hybrids were performed as described by Van Landschoot and De Ley (40). Each DNA-rRNA hybrid is characterized by its $T_{m(e)}$ value (the melting temperature of elution), the temperature at which 50% of the DNA-rRNA hybrid is denatured. The higher the $T_{m(e)}$ of a heterologous hybrid, the closer both strains are related.

DNA-DNA hybridization experiments. The degree of DNA-DNA binding, expressed as a percent, was determined spectrophotometrically by the initial renaturation rate method of De Ley et al. (8). Each value given is the average of at least two hybridization experiments. Values of 30% DNA binding and less do not represent significant DNA homology. The total DNA concentration was 65 µg/ml, and the optimal renaturation temperature in 2× SSC (sodium saline citrate; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7) was 80.7°C.

Fatty acid methyl ester analysis. After an incubation period of 24 h on Trypticase soy agar at 35°C (catalog no. 11768; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.), a loopful of well-grown cells was harvested and fatty acid methyl esters were prepared, separated, and identified by using the Microbial Identification System (Microbial ID, Inc., Newark, Del.) as described before (39).

Conventional biochemical tests. All saccharolytic strains were characterized in a range of 68 conventional biochemical tests by methods described previously (14); nonsaccharolytic strains were characterized in a limited range of these tests.

RESULTS

PAGE of whole-cell proteins. Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation level between duplicate protein patterns was more than 93%.

The whole-cell protein patterns of several *Burkholderia* strains were often characterized by a distortion of part of the banding pattern. This "smiling" appearance is probably due to the presence of polysaccharides which are not adequately removed during the extraction procedure and was a reproducible phenomenon, as repeated extractions resulted in similar distortions. It was essential not to exceed the amount of 80 to 90 mg of wet cell weight for the extraction of the cellular proteins (see the procedure described by Pot et al. [25]). When present, distortions were mostly seen in the 45,000- to 66,000-molecular-weight region (data not shown) and influenced the correlation level between protein patterns. It was thus essential to

thoroughly compare the clustering result of the numerical analysis of the protein patterns (Fig. 1) with the profiles themselves in order to significantly delineate clusters of strains with similar protein patterns.

After extensive examination of the protein profiles and the dendrogram, 12 clusters were delineated (Fig. 1). Several strains occupied a separate position in the dendrogram. Reference strains and recent isolates of *B. cepacia* constituted eight distinct clusters: i, v, vi, vii, viii, x, xi, and xii.

Cluster i comprises 10 *B. cepacia* reference strains (including the type strain), the *P. pyrrocinia* type strain, a single cystic fibrosis isolate (LMG 14095), 6 strains from the hospital environment, 2 strains from non-cystic fibrosis patients, and 4 strains from plant rhizospheres or bark. *P. pyrrocinia* LMG 14191^T and strain LMG 14086 group at the edge of this cluster. Cluster ii comprises two subclusters and the *B. coveneniensis* type strain, which occupies a separate position. Subclusters iia and iib comprise the three reference strains of *B. gladioli* and *B. glumae*, respectively. Cluster iii contains the *P. aeruginosa* reference strains. Cluster iv comprises the *B. plantarii* reference strains and *B. vandii*, which have indistinguishable protein patterns. *Ralstonia eutropha* LMG 1199^T, *B. andropogonis* LMG 2129^T, *B. caryophylli* LMG 2155^T, and cluster v (*B. cepacia* LMG 2161 and LMG 14087) group near cluster iv, but each occupies a separate position. Cluster vi is composed of five *B. cepacia* strains which were isolated from three Belgian cystic fibrosis patients. Cluster vii comprises a *B. vietnamiensis* reference strain (LMG 6998), two strains from Swedish cystic fibrosis patients which were originally identified as *B. cepacia*, and a soil isolate. Similarly, cluster viii comprises three *B. vietnamiensis* reference strains (including the type strain) and a single soil isolate originally identified as *B. cepacia*. In addition, *B. vietnamiensis* LMG 6999 occupies a separate position in the dendrogram. The two *P. glathei* reference strains constitute cluster ix. Cluster x comprises 15 *B. cepacia* strains. Twelve isolates were obtained from four cystic fibrosis patients (three from Belgium and one from the United Kingdom), one was isolated from a hospital flower vase, one from soil, and one from a non-cystic fibrosis patient. Cluster xi comprises four isolates obtained from three cystic fibrosis patients (one from Belgium and two from the United Kingdom). Cluster xii comprises 47 *B. cepacia* isolates obtained from 41 cystic fibrosis patients (6 from Belgium and 35 from the United Kingdom) and two lung transplant patients (we do not know if the latter patients are cystic fibrosis patients or not). Three isolates from Belgium and one from the United Kingdom constitute a first subcluster (xiia), while the remaining strains constitute subcluster xiib. Strain *B. cepacia* LMG 14939 occupies a separate position near cluster xii. Finally, the *Ralstonia pickettii* and *Ralstonia solanacearum* type strains each occupy separate positions in the dendrogram.

DNA-rRNA hybridization experiments. The DNA-rRNA hybridization results are listed in Table 2. *B. cepacia* strains from clusters i, v, vi, x, xi, and xii all exhibit high $T_{m(e)}$ values (79.0 to 81.6°C) versus *B. cepacia* LMG 1222^T rRNA. *B. coveneniensis* LMG 11626^T occupies a more distinct position, somewhat lower on the *B. cepacia* rRNA branch ($T_{m(e)}$, 77.7°C). *P. pyrrocinia*, and *P. glathei* reference strains also belong to the *B. cepacia* rRNA branch, with $T_{m(e)}$ values similar to those of *B. caryophylli* and *B. andropogonis*, respectively (Table 2).

DNA-DNA hybridization experiments. The results of the DNA-DNA hybridizations are summarized in Table 3. Within the group of *B. cepacia* strains examined, five major genotypic subgroups can be delineated. One of these genotypic subgroups was identified as *B. vietnamiensis*; the others are re-

TABLE 1. List of strains studied

Name as received and strain designation (revised name) ^a	Genomovar	Type of analysis ^b	Other strain designation(s) ^c	Source ^d
<i>B. andropogonis</i> LMG 2129 ^T		f	NCPPB 934 ^T	<i>Sorghum bicolor</i>
<i>B. caryophylli</i> LMG 2155 ^T		f	NCPPB 2151 ^T	<i>Dianthus caryophyllus</i>
<i>B. cepacia</i> reference strains: LMG 1222 ^T	I	i, f, b	Palleroni 717 ^T , NCTC 10743 ^T	<i>Allium cepa</i>
LMG 6863	I	i, f	NCIB 9690	River Maraval (Trinidad)
LMG 6963	I	i, f	PDDCC 5981	Soil (Australia)
LMG 6964	I	i, f, b	PDDCC 5982	<i>Lycopersicum lycopersicum</i> (L.) (Australia)
LMG 6981	I	i, f, b	ATCC 25609, NCTC 10744	Bronchial washing
LMG 6986	I	i, f, b	CCUG 788	Urine
LMG 6988	I	i, f, b	CCUG 1603	Leg wound (Sweden, 1972)
LMG 6992	I	i, f, b	CCUG 2857	Soil (Trinidad, 1960)
LMG 6997	I	i, f, b	CCUG 3461B	Ear (Sweden, 1974)
LMG 7000	I	i, f, b	CCUG 13348	Blood (Sweden, 1983)
LMG 2161	I	v	NCTC 10661	Forest soil (Trinidad)
LMG 17588 (<i>B. multivorans</i>)	II	x	ATCC 17616	Soil (United States)
<i>B. cepacia</i> field isolates:				
LMG 13014	I	i, f		Hand cream (Belgium, 1991)
LMG 13015	I	i, f		Humidifier (Belgium, 1991)
LMG 13016	I	i, f		Catheter (Belgium, 1991)
LMG 13017	I	i, f		Blood culture (Belgium, 1988)
LMG 13018	I	i, f		Blood culture (Belgium, 1989)
LMG 14086	I	i, f, b		Respirator (United Kingdom, 1970)
LMG 14095	I	i, f, b		Cystic fibrosis patient (United Kingdom, 1974)
LMG 15950	I	i, f		Water bath, cystic fibrosis ward (Belgium, 1994)
LMG 15951	I	i, f		Spirometer (Belgium, 1994)
LMG 16669	I	i	J2541	Rhizosphere of <i>Epidendrum o'bienianum</i> (United Kingdom)
LMG 16670	I	i	J2552	Rhizosphere of <i>Carludovica palmata</i> (United Kingdom)
LMG 16671	I	i	J2535	Rotting bark, orchid house (United Kingdom)
LMG 16672	I	i	J2540	Rhizosphere of banana plant (United Kingdom)
LMG 14087	I	v, f, b		Wound swab (United Kingdom, 1988)
LMG 13010 ^T (<i>B. multivorans</i>)	II	x, f, b	NCTC 13007 ^T	Cystic fibrosis patient (Belgium, 1992)
LMG 14273 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14275 (<i>B. multivorans</i>)	II	x, f		Cystic fibrosis patient (Belgium, 1993)
LMG 14277 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14280 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14281 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14282 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14284 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14286 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14293 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14296 (<i>B. multivorans</i>)	II	x, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 16660 (<i>B. multivorans</i>)	II	x, f	C1576	Cystic fibrosis patient (United Kingdom), Glasgow epidemic reference
LMG 16665 (<i>B. multivorans</i>)	II	x, f	C1962	Non-cystic fibrosis patient, brain abscess (United Kingdom)
LMG 16668 (<i>B. multivorans</i>)	II	x, f	J2395	Hospital flower vase (United Kingdom)
LMG 13053	III	xi, f		Cystic fibrosis patient (Belgium, 1987)
LMG 14274	III	xi, f		Cystic fibrosis patient (Belgium, 1993)
LMG 16661	III	xi, f		Cystic fibrosis patient (United Kingdom)
LMG 16662	III	xi, f		Cystic fibrosis patient (United Kingdom)
LMG 14270	III	xiii, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14271	III	xiii, f		Cystic fibrosis patient (Belgium, 1993)
LMG 14942	III	xiii, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 16659	III	xiii	C1394	Cystic fibrosis patient (United Kingdom), Manchester epidemic reference
LMG 12614	III	xiib, f, b	NCTC 13010	Cystic fibrosis patient (United Kingdom, 1992)
LMG 12615	III	xiib, f, b	NCTC 13008	Cystic fibrosis patient (United Kingdom, 1992)

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TABLE 1—Continued

Name as received and strain designation (revised name) ^a	Genomovar	Type of analysis ^b	Other strain designation(s) ^c	Source ^d
LMG 13011	III	xiib, f		Cystic fibrosis patient (Belgium, 1988)
LMG 13012	III	xiib, f		Cystic fibrosis patient (Belgium, 1988)
LMG 13054	III	xiib, f		Cystic fibrosis patient (Belgium, 1987)
LMG 13307	III	xiib, f, b	ISL 866/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13308	III	xiib, f, b	ISL 101/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13309	III	xiib, f, b	ISL 464/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13310	III	xiib, f, b	ISL 1021/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13311	III	xiib, f, b	ISL 87/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13312	III	xiib, f, b	ISL 864/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13313	III	xiib, f, b	ISL 878/92	Lung transplant patient (United Kingdom, 1992)
LMG 13314	III	xiib, f, b	ISL 7/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13315	III	xiib, f, b	ISL 854/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13316	III	xiib, f, b	ISL 856/91	Cystic fibrosis patient (United Kingdom, 1991)
LMG 13317	III	xiib, f, b	ISL 865/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13318	III	xiib, f, b	ISL 921/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13319	III	xiib, f, b	ISL 857/91	Cystic fibrosis patient (United Kingdom, 1991)
LMG 13320	III	xiib, f, b	ISL 863/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13321	III	xiib, f, b	ISL 860/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13322	III	xiib, f, b	ISL 832/91	Cystic fibrosis patient (United Kingdom, 1991)
LMG 13323	III	xiib, f, b	ISL 857/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13324	III	xiib, f, b	ISL 752/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13325	III	xiib, f, b	ISL 182/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13326	III	xiib, f, b	ISL 84/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13327	III	xiib, f, b	ISL 880/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13328	III	xiib, f, b	ISL 86/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13329	III	xiib, f, b	ISL 11/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13330	III	xiib, f, b	ISL 41/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13331	III	xiib, f, b	ISL 85/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 13332	III	xiib, f, b	ISL 862/92	Cystic fibrosis patient (United Kingdom, 1992)
LMG 14088	III	xiib, f, b	ISL 1/93	Cystic fibrosis patient (United Kingdom, 1993)
LMG 14089	III	xiib, f, b	ISL 4/93	Cystic fibrosis patient (United Kingdom, 1993)
LMG 14090	III	xiib, f, b	ISL 5/93	Cystic fibrosis patient (United Kingdom, 1993)
LMG 14091	III	xiib, f, b	ISL 54/93	Cystic fibrosis patient (United Kingdom, 1993)
LMG 14092	III	xiib, f, b	ISL 127/93	Cystic fibrosis patient (United Kingdom, 1993)
LMG 14093	III	xiib, f, b	ISL 128/93	Cystic fibrosis patient (United Kingdom, 1993)

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TABLE 1—Continued

Name as received and strain designation (revised name) ^a	Genomovar	Type of analysis ^b	Other strain designation(s) ^c	Source ^d
LMG 14094	III	xiib, f, b	ISL 356/93	Lung transplant patient (United Kingdom, 1993)
LMG 14276	III	xiib, f, b	NCTC 13009	Cystic fibrosis patient (Belgium, 1993)
LMG 14937	III	xiib, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14941	III	xiib, f, b		Cystic fibrosis patient (Belgium, 1994)
LMG 16656	III	xiib	J2315	Cystic fibrosis patient (United Kingdom, 1989), ET12 reference
LMG 16658	III	xiib	C1632	Cystic fibrosis patient (United Kingdom), Newcastle epidemic reference
LMG 14291	IV	vi, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14294	IV	vi, f, b	NCTC 13011	Cystic fibrosis patient (Belgium, 1993)
LMG 14295	IV	vi, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14940	IV	vi, f, b		Cystic fibrosis patient (Belgium, 1994)
LMG 15949	IV	vi, f, b		Cystic fibrosis patient (Belgium, 1993)
LMG 14939		f		Cystic fibrosis patient (Belgium, 1994)
LMG 10824 (<i>B. vietnamiensis</i>)		vii		Rice root (Indonesia)
LMG 16230 (<i>B. vietnamiensis</i>)		vii, f, b	CCUG 34416	Cystic fibrosis patient (Sweden)
LMG 16232 (<i>B. vietnamiensis</i>)		vii, f, b	CCUG 31370	Cystic fibrosis patient (Sweden)
LMG 10823 (<i>B. vietnamiensis</i>)		viii		Soil (Indonesia)
<i>B. cocovenenans</i> LMG 11626 ^T		ii, f	LMD 38.18 ^T	Poisoned bongkre (Java)
<i>B. gladioli</i> LMG 2216 ^T		ii, f, b	ATCC 10248 ^T	<i>Gladiolus</i> sp. (United States)
<i>B. gladioli</i> LMG 6881		ii, f	NCPPB 1051	<i>Gladiolus</i> sp. (Zimbabwe)
<i>B. gladioli</i> LMG 6882		ii, f	NCPPB 1887	<i>Gladiolus</i> sp. (United States)
<i>B. glumae</i> LMG 1277		ii, f	NCPPB 2391	<i>Oryza sativa</i> (Japan)
<i>B. glumae</i> LMG 2196 ^T		ii, f	NCPPB 2981 ^T	<i>Oryza sativa</i> (Japan)
<i>B. glumae</i> LMG 6952		ii, f	PDDCC 3728	<i>Oryza sativa</i> (Japan)
<i>B. plantarii</i> LMG 9035 ^T		iv, f	ICMP 9424 ^T	<i>Oryza sativa</i> (Japan)
<i>B. plantarii</i> LMG 10907		iv, f	ICMP 9425	Soil (Japan)
<i>B. plantarii</i> LMG 10908		iv, f	ICMP 9426	<i>Oryza sativa</i> (Japan)
<i>B. vandii</i> LMG 16020 ^T		iv, f	JCM 7957 ^T	<i>Vanda</i> sp. (Japan)
<i>B. vietnamiensis</i> LMG 6998		vii, f, b	CCUG 7246	Blood (Sweden, 1978)
<i>B. vietnamiensis</i> LMG 10926		viii, f, b	TVV 69	Rice rhizosphere (Vietnam)
<i>B. vietnamiensis</i> LMG 10929 ^T		viii, f, b	TVV 75 ^T	Rice rhizosphere (Vietnam)
<i>B. vietnamiensis</i> LMG 10930		viii, f, b	TVV 116	Rice rhizosphere (Vietnam)
<i>B. vietnamiensis</i> LMG 6999		f, b	CCUG 9631	Neck abscess, child (1980)
<i>P. aeruginosa</i> LMG 1242 ^T		iii	CCEB 481 ^T	
<i>P. aeruginosa</i> LMG 5827		iii	NCPPB 1966	Lung
<i>P. aeruginosa</i> LMG 6395		iii	DSM 1117	Blood culture
<i>P. glathei</i> LMG 14190 ^T (<i>B. glathei</i>)		ix, f	ATCC 29195 ^T	Soil (Germany)
<i>P. glathei</i> LMG 14932 (<i>B. glathei</i>)		ix, f	ATCC 29198	Soil (Germany)
<i>P. pyrrocinia</i> LMG 14191 ^T (<i>B. pyrrocinia</i>)		i, f	ATCC 15958 ^T	Soil
<i>R. eutropha</i> LMG 1199 ^T			ATCC 17697 ^T	Soil (United States)
<i>R. pickettii</i> LMG 5942 ^T			CCUG 3318 ^T	Human (United States)
<i>R. solanacearum</i> LMG 2299 ^T			NCPPB 325 ^T	<i>Lycopersicon lycopersicum</i> (L.) (United States)

^a T, type strain.^b i through xii, protein electrophoresis (the letters refer to positions in the protein electrophoretic dendrogram [Fig. 1]); f, fatty acid analysis; b, biochemical tests. ^c ATCC, American Type Culture Collection, Rockville, Maryland; CCUG, Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Saitama, Japan; LMD, Laboratorium voor Microbiologie, Technische Universiteit, Delft, The Netherlands; LMG, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Ghent, Belgium; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden Laboratory, Hertfordshire, United Kingdom; NCTC, National Collection of Type Cultures, London, United Kingdom; PDDCC, Plant Diseases Division Culture Collection, DSIR Mount Albert Research Center, Auckland, New Zealand.^d If known.

ferred to as genomovars I through IV (see below). Strains from electrophoretic clusters i and v form a single genotypic subgroup with DNA-DNA binding values above 58% (genomovar I), while hybridization values towards representatives of other subgroups are between 16 and 45%. Strains of electrophoretic cluster x share DNA-DNA binding values above 82% (genomovar II), while values towards representatives of other subgroups are between 19 and 54%. The two strains of electro-

phoretic cluster vi have a DNA-DNA binding value of 100% (genomovar IV), while values towards representatives of other groups are between 23 and 54%. Strains of electrophoretic clusters xi and xii form a single genotypic subgroup sharing DNA-DNA binding values between 54 and 100% (genomovar III), while values towards representatives of other subgroups are between 18 and 54%. Finally, *B. vietnamiensis* strains of clusters vii and viii share a DNA-DNA binding value of 86%,

TABLE 3. DNA base ratio and DNA-DNA binding values of all strains examined

Strain	Protein electro- phoretic cluster (genomovar)	G+C content (mol%)	DNA-DNA binding value (%) with strain:																					
			LMG 1222 ^T	LMG 14087	LMG 6988	LMG 13010	LMG 14273	LMG 14275	LMG 14293	LMG 14294	LMG 14940	LMG 12614	LMG 12615	LMG 13327	LMG 13011	LMG 14271	LMG 14274	LMG 13053	LMG 16661	LMG 14939	LMG 10929 ^T	LMG 6998	LMG 11626 ^T	LMG 14191 ^T
<i>B. cepacia</i> LMG 1222 ^T	i (I)	67	100																					
<i>B. cepacia</i> LMG 14087	v (I)	67	91	100																				
<i>B. cepacia</i> LMG 6988	i (I)	68	58		100																			
<i>B. cepacia</i> LMG 13010	x (II)	68	24			100																		
<i>B. cepacia</i> LMG 14273	x (II)						100																	
<i>B. cepacia</i> LMG 14275	x (II)	68	42			100	82	100																
<i>B. cepacia</i> LMG 14293	x (II)						88																	
<i>B. cepacia</i> LMG 14294	vi (IV)	68		35				54	31	100														
<i>B. cepacia</i> LMG 14940	vi (IV)	69	41					24	100	100														
<i>B. cepacia</i> LMG 12614	xia (III)	67	33								100													
<i>B. cepacia</i> LMG 12615	xia (III)	68	42			36				54	89	100												
<i>B. cepacia</i> LMG 13320	xia (III)	67					32			43			100											
<i>B. cepacia</i> LMG 13327	xia (III)	68				19							93	100										
<i>B. cepacia</i> LMG 13011	xia (III)	68	33										54	58	100									
<i>B. cepacia</i> LMG 14271	xib (III)									49						100								
<i>B. cepacia</i> LMG 14274	xi (III)																100							
<i>B. cepacia</i> LMG 13053	xi (III)	68		45		42		40					76		99			100						
<i>B. cepacia</i> LMG 16661	xi (III)												69		89				100					
<i>B. cepacia</i> LMG 14939	xi (III)								47				50	42		35	57	56		100				
<i>B. vietnamiensis</i> LMG 10929 ^T	viii	68		34		40		31		26							42				100			
<i>B. vietnamiensis</i> LMG 6998	vii	68	16				30		23		18	23									86	100		
<i>B. cocovenenans</i> LMG 11626 ^T	ii	69	13			15		14					11								31		100	
<i>B. gladioli</i> LMG 2216 ^T	iii	11					4		24	1	12	13				23							100	
<i>B. pyrrocinia</i> LMG 14191 ^T	i	59	59				31						55		63		54				56			100

genotypic subgroups within *B. cepacia*, *B. vietnamiensis*, and *B. gladioli*. In addition, phenotypic data for 11 *B. gladioli* strains (these strains are not included in Table 1) isolated from cystic fibrosis patients (5) and identified by DNA-DNA hybridization experiments were included for comparison.

The strains of *B. cepacia* genomovar III proved very heterogeneous in their phenotypic characters, and a number of nonsaccharolytic variants were observed. The saccharolytic strains produced acid from various numbers of carbohydrates but needed to be divided into two biovars to facilitate practical identification. The nonsaccharolytic strains represent a third biovar. The results for strain LMG 14088 are not presented because this strain gave an oxidative reaction in glucose oxidation-fermentation (OF) medium but produced acid only from glucose in ammonium salt medium; it therefore did not correspond with any of the three biovars recognizable within genomovar III. The most useful characters for differentiating the four genomovars of *B. cepacia*, including the three biovars of genomovar III, and of *B. vietnamiensis* and *B. gladioli* are shown in Table 5.

DISCUSSION

B. cepacia, traditionally a pathogen in onions (22), has been recognized as an important pathogen in chronic lung disease in patients with cystic fibrosis. A number of observations pointed to heterogeneity among cystic fibrosis isolates of this species and prompted us to collect strains from various sources and from different geographical areas in order to compose a representative collection for a taxonomic study.

Well-characterized reference strains from plants, water, soil, and humans (non-cystic fibrosis patients) and 89 recent isolates identified as *B. cepacia* by means of biochemical tests, cellular fatty acid analysis, or both were included. These 89 strains were isolated primarily from cystic fibrosis patients (70 strains isolated from patients in Belgium, Sweden, and the United Kingdom); 2 strains were from lung transplant patients; 4 strains were from non-cystic fibrosis patients; 7 strains were from the hospital environment (4 of these were obtained from material or rooms used by cystic fibrosis patients); and 6 strains were isolated from tropical plants or soil.

Representative strains of all of the other *Burkholderia* species and of *P. aeruginosa*, *R. solanacearum*, *R. pickettii*, and *R. etrophia* were included as references.

Detection of marked heterogeneity among *B. cepacia* strains. SDS-PAGE of whole-cell proteins and computer-assisted comparisons of their protein patterns are often used to compare large numbers of bacteria and to group closely related strains (6). In general, strains with highly similar protein patterns share high DNA-DNA binding values and thus belong to a single genomic species. However, within several species, multiple protein electrophoretic subgroups have been described and are considered electrophoretic types (6). In numerical analyses, electrophoretic types of a single species may form distinct clusters, and therefore the precise relationships between strains belonging to different clusters in a dendrogram (Fig. 1) have to be established by means of DNA-DNA hybridization studies.

In the present numerical comparison of whole-cell protein patterns, *B. cepacia* reference strains and recent isolates constituted eight distinct clusters (Fig. 1), which were clearly distinct from the other reference taxa examined (i.e., various other *Burkholderia* species, *Ralstonia* species, and *P. aeruginosa*) (Fig. 1). Six of the *B. cepacia* reference strains of protein electrophoretic cluster i (LMG 1222^T, LMG 6981, LMG 6988, LMG 6963, LMG 6964, and LMG 6997) were previously included in a DNA-DNA hybridization study and shared DNA-DNA binding values of more than 65% (10), confirming that

TABLE 4. Fatty acid compositions of the strains studied^a

Fatty acid	Amt (%) in														
	<i>B. cepacia</i> genomovar I, cluster j ^b (19) ^c	<i>B. cepacia</i> genomovar II, cluster x (14)	<i>B. cepacia</i> genomovar IV, cluster vi (5)	<i>B. cepacia</i> genomovar III, cluster xii (44)	<i>B. cepacia</i> genomovar III, cluster xi (4)	<i>B. pyrocinia</i> LMG 14191 ^{Td}	<i>B. vietnamiensis</i> (7)	<i>B. gladioli</i> (3)	<i>B. cocovenerans</i> LMG 11626 ^T	<i>B. glumae</i> (3)	<i>B. plantarii</i> (3)	<i>B. vandii</i> LMG 16020 ^T	<i>B. andropogonis</i> LMG 2129 ^{Te}	<i>B. caryophylli</i> LMG 2155 ^T	<i>B. glutinei</i> (2)
14:0	4.0 ± 1.5	4.8 ± 0.6	4.9 ± 0.2	4.3 ± 0.7	4.1 ± 0.5	Tr	3.8 ± 0.6	5.0 ± 0.2	4.9	4.7 ± 0.1	4.5 ± 0.1	5.8	4.7	3.9	4.3 ± 0.1
16:1 ω7c	4.6 ± 2.5	6.5 ± 2.5	2.7 ± 0.8	3.3 ± 1.6	2.7 ± 0.3	7.3	9.8 ± 2.5	5.1 ± 2.3	2.5	4.5 ± 0.2	1.2 ± 0.4	1.0	6.2	12.1	13.0 ± 3.8
16:0	25.9 ± 3.2	28.9 ± 3.5	28.0 ± 4.6	26.6 ± 3.3	25.6 ± 3.7	22.9	19.5 ± 2.5	29.2 ± 1.2	28.3	27.0 ± 2.4	33.6 ± 2.7	38.3	17.2	21.3	17.5 ± 2.5
17:0 cyclo	17.3 ± 3.4	18.2 ± 4.9	20.1 ± 2.7	22.5 ± 4.3	20.1 ± 4.0	13.5	14.0 ± 4.9	16.9 ± 2.1	18.2	11.8 ± 0.7	25.2 ± 0.1	23.2	9.9	2.7	12.1 ± 0.5
16:1 2OH	1.1 ± 0.6 (17) ^f	1.3 ± 0.8 (12)	Tr (2)	1.5 ± 1.0 (38)	1.0 ± 0.2	1.9	1.3 ± 0.2	1.5 ± 0.3	1.5	1.1 ± 0.2	Tr	ND	1.7	3.7	Tr
16:0 2OH	4.0 ± 1.4	2.8 ± 1.0	3.4 ± 1.3	2.9 ± 0.9	3.4 ± 0.9	4.4	2.8 ± 0.4	3.1 ± 0.3	3.5	3.0 ± 1.2	1.4 ± 0.1	1.1	6.2	5.3	2.5 ± 1.8
16:0 3OH	6.3 ± 1.0	6.8 ± 0.5	5.9 ± 0.5	6.0 ± 1.0	7.3 ± 2.0	5.8	6.4 ± 0.7	7.0 ± 1.2	7.4	5.6 ± 0.4	6.3 ± 0.5	7.4	3.8	5.9	6.2 ± 1.2
18:1	11.8 ± 3.5	11.5 ± 4.8	10.7 ± 1.8	9.4 ± 5.0	9.1 ± 3.8	22.2	19.7 ± 5.5	11.0 ± 3.7	6.0	22.2 ± 1.7	5.3 ± 1.3	4.9	14.2	32.3	32.6 ± 0.6
18:0	Tr	Tr (5)	Tr (2)	2.4 ± 2.2 (42)	Tr (3)	Tr	5.2 ± 3.4	Tr	1.1	Tr	1.2 ± 0.1	1.4	ND	1.0	Tr
19:0 cyclo ω8c	13.1 ± 4.6	9.7 ± 3.3	16.1 ± 4.7	12.0 ± 4.2	14.7 ± 5.5	4.7	5.8 ± 2.5	8.9 ± 1.9	13.3	8.5 ± 1.1	10.3 ± 2.1	9.3	28.6	1.4	2.5 ± 0.6
18:1 2OH	2.4 ± 0.7	1.3 ± 0.7 (12)	2.3 ± 0.7	1.5 ± 1.4 (41)	3.0 ± 0.6	3.2	3.4 ± 0.5	3.5 ± 0.7	5.7	3.5 ± 0.3	4.4 ± 2.0	2.6	1.4	3.7	Tr (1)
14:0 3OH	6.0 ± 1.4	6.4 ± 1.1	5.1 ± 0.5	6.1 ± 1.0	6.4 ± 1.9	7.3	6.3 ± 0.9	6.8 ± 1.1	7.0	6.1 ± 0.6	5.2 ± 0.8	4.9	4.0	6.1	7.5 ± 0.5

^a 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%); ND, not detected.

^b Protein electrophoretic cluster numbers (Fig. 1).

^c Numbers in parentheses indicate numbers of strains examined.

^d *B. pyrocinia* LMG 14191^T also contains 4.2% 12:0.

^e *B. andropogonis* LMG 2129^T also contains 1.2% 13:1 AT 12-13.

^f The number of strains comprising the fatty acid is given only when not present in all of the strains examined.

TABLE 5. Biochemical differentiation of genomovars of *B. cepacia*, *B. vietnamiensis*, and *B. gladioli*

Character	Result ^a for:						<i>B. gladioli</i> (12)	<i>B. vietnamiensis</i> (7)
	<i>B. cepacia</i> genomovar							
	I (11)	II ^b (10)	III, biovar			IV (5)		
a (8)			b (6)	c (25)				
Acid production in ammonium salt medium from:								
Glucose	+	+	+	+	—	+	+	+
Adonitol	+	+	+	—	—	+	+	—
Cellobiose	+	+	+	5	—	+	7	+
Dulcitol	+	+	+	—	—	+	+	+
Fructose	+	+	+	—	2/25	+	+	+
Inositol	+	+	+	2	—	+	+	+
Lactose	+	+	+	+	—	4	—	+
Maltose	+	+	7	2	—	+	—	+
Mannitol	+	+	+	—	—	+	+	+
Raffinose	9 ^b	—	—	—	—	—	—	5
Salicin	10	9	4	—	—	—	—	—
Sorbitol	+	+	+	—	—	+	+	+
Sucrose	7	—	+	+	—	—	—	+
Xylose	+	+	+	5	—	+	+	+
Acid from glucose peptone water sugar	10	+	+	5	—	+	—	+
Acid from 10% (wt/vol):								
Glucose	+	+	+	+	—	+	10	+
Lactose	+	+	+	+	—	+	1	+
Alkaline reaction in OF medium	—	—	—	—	22/25	—	—	—
Casein digestion	10	—	4	4	—	4	11	—
Growth at 42°C	—	+	+	5	+	—	—	—
Growth on cetrimide agar	+	+	+	+	+	+	2	+
Growth on Simmons citrate	+	+	+	1	4/25	+	+	+
Malonate utilization	+	9	4	3	1/12	+	—	+
Melanin-like pigment on tyrosine agar	—	—	7	3	1/25	—	1	—
Motility at 37°C	3	+	+	4	16/23	+	—	+
Nitrate reduction	4	+	—	3	21/25	3	5	5
Oxidative in OF medium	+	+	+	+	—	+	11	+
Urease production	10	+	2	—	1/25	3	9	+

^a Numbers in parentheses are numbers of strains. +, all strains (for *B. cepacia* genomovar III, biovar c, all strains tested) positive; —, all strains (for *B. cepacia* genomovar III, biovar c, all strains tested) negative. The numbers in the field are the numbers of strains giving a positive result; for *B. cepacia* genomovar III, biovar c, the ratios are numbers of strains giving a positive result/numbers of strains tested.

^b Proposed *B. multivorans*.

they constitute a single genomic group corresponding to a species as defined by Wayne et al. (41). Two of these strains were included in the present DNA-DNA hybridization study, and a binding value of 58% was found (Table 3). In addition, one of the two *B. cepacia* strains from cluster v (LMG 14087) also showed a high hybridization value (91%) towards the type strain (LMG 1222), indicating that both clusters represent different electrophoretic types of one species (Fig. 1; Table 3).

Similarly, *B. cepacia* strains of protein electrophoretic clusters xi and xii shared high DNA-DNA hybridization values (average value: 82 ± 14%; Table 3) and thus constitute a single species. Strains of protein electrophoretic clusters vi and x also formed homogeneous groups. Finally, two *B. vietnamiensis* strains representing electrophoretic clusters vii and viii shared high DNA-DNA hybridization values, confirming a previous report (10).

Hybridizations between representatives of these five genomic groups yielded clearly lower hybridization values, mostly in the range of 20 to 45% (Table 3), and none of the

strains shared high DNA-DNA binding values with *B. gladioli* or *B. coccovenans*, which are close relatives of *B. cepacia*. In the absence of differential classical phenotypic tests, members of this *B. cepacia* complex were previously referred to as genomovar I (clusters i and v), genomovar II (cluster x), genomovar III (clusters xi and xii), genomovar IV (cluster vi), and *B. vietnamiensis* (clusters vii and viii) (13, 37) in accordance with the guidelines and recommendations for the delineation of new species (36, 41). These five major genomic groups (including *B. vietnamiensis*) correspond to five distinct species, each with a similar DNA base ratio of approximately 68 mol% (Table 3). Finally, the separate position of strain *B. cepacia* LMG 14939 in the protein electrophoretic dendrogram was confirmed by the DNA-DNA hybridization analysis because values between 31 and 57% were measured towards representatives of *B. cepacia* genomovars I through IV.

Hybridizations between DNA of representative strains of each of these five taxa and rRNA of *B. cepacia* LMG 1222^T confirm that they all cluster at the top of the *B. cepacia* rRNA

branch (Table 2). The 16S rRNA sequence of strain LMG 17588 (=ATCC 17616), a typical genomovar II strain, was reported to exhibit more than 98% similarity with the 16S rRNA sequences of *B. cepacia* genomovar I strains (LMG 1222^T [=ATCC 25416^T] and LMG 2161 [=ATCC 17759]) and of *B. vietnamiensis* LMG 10929^T (=TVV 75^T) (20). *B. gladioli* occupies a distinct position, clearly lower on the *B. cepacia* rRNA branch (Table 2) (10, 20). *B. glumae*, *B. plantarii*, *B. cocovenenans*, and *B. mallei* are its nearest neighbors (Table 2) (10).

An analysis of 68 conventional biochemical characteristics (14) yielded a number of tests to differentiate between these genomovars, *B. vietnamiensis*, and *B. gladioli*, although occasionally strains could be found which do not fit into the expected pattern (Table 5). From these data it appears that particularly *B. vietnamiensis*, *B. gladioli*, and *B. cepacia* genomovar II strains are identifiable. The biochemical differentiation of *B. cepacia* genomovars I, III, and IV is less clear, and the identification of *B. cepacia* genomovar III can only be achieved by identifying these isolates at the biovar level. Nonsaccharolytic strains of *B. cepacia* (biovar c) are likely to be confused with *Alcaligenes* or *Comamonas* strains and were reported earlier by Pitt et al. (24), who demonstrated that saccharolytic and nonsaccharolytic isolates may belong to the same ribotype. Recognition of these nonsaccharolytic strains as *B. cepacia*-like strains was achieved by observing their ability to grow on *B. cepacia*-selective medium, the presence of cytochrome oxidase production, and the absence of arginine hydrolysis (24). Marked phenotypic variability among strains with the same ribotype was also reported by Larsen et al. (18).

There are clearly five genomic species within strains routinely identified as *B. cepacia*. These are mostly distinguishable phenotypically, but occasional atypical characters mean that unequivocal identification is not possible in all cases. At present, we feel there is sufficient biochemical information to propose a species name for genomovar II strains but not for the others. We propose to continue to use the terms *B. cepacia* genomovars I, III, and IV for these taxa pending further study.

***P. pyrracinia* and *P. glathei*.** The taxonomic affiliations of a number of *Pseudomonas* species have not been fully determined (16). The combined evidence of DNA-rRNA hybridization experiments (Table 2), fatty acid data (the high ratio of the 16:0 3OH percentage to the total percentage of 3-hydroxy fatty acids [10]), and DNA-DNA hybridizations indicates that both *P. pyrracinia* and *P. glathei* belong to the genus *Burkholderia* (10).

In the present study, *P. pyrracinia* LMG 14191^T could be distinguished from the other taxa examined by its characteristic fatty acid profile but was indistinguishable from *B. cepacia* genomovar I strains in whole-cell protein electrophoresis. Below, we propose to transfer this species into the genus *Burkholderia* as *B. pyrracinia* comb. nov.

In addition, the DNA base ratio (65% for the type strain [21]) and the general phenotypic and chemotaxonomic profiles of *P. glathei* conform to those of *Burkholderia* species. Whole-cell protein and fatty acid analyses are useful to differentiate this species from other *Burkholderia* species (Fig. 1; Table 4). Interestingly, the genomes of *P. glathei* strains are composed of multiple chromosomes, a highly unusual characteristic which is present in several other members of the *Burkholderia*-*Ralstonia* lineage (20). We conclude that *P. glathei* should be transferred to the genus *Burkholderia* as *B. glathei* comb. nov.

Application of whole-cell fatty acid analysis for the differentiation of *Burkholderia* species. Several investigators examined the whole-cell fatty acid components of *Burkholderia* strains; this technique is often used for the identification of *B.*

cepacia strains. When comparing the results of different fatty acid analyses, it is important to realize that the quantitative composition may be strongly influenced by various factors including growth medium, time, and temperature. Urakami et al. (35) examined several *Burkholderia* species and could not detect differences in their fatty acid patterns, whereas Yabuuchi et al. (43) reported clear differences between the type strains of several species. In both studies, only one or two reference strains per species were included. Stead (31) examined several strains per species and presented the average value and standard deviation per taxon. *B. cepacia*, *B. gladioli*, and some of the *B. glumae* strains had indistinguishable profiles; other *Burkholderia* species were readily differentiated (31). In our study (Table 4), the *B. cepacia* genomovars and *B. gladioli* were indistinguishable. Only a single *B. cocovenenans* strain was examined, and its pattern was very similar to those of the *B. cepacia* and *B. gladioli* strains. In addition, the *B. vandii* and *B. plantarii* strains were characterized by a lower percentage of 18:1, a higher percentage of 16:0, and a lower ratio of 16:1 ω 7c/17:0 cyclo compared to *B. cepacia* and *B. gladioli* strains. However, considering all standard deviations, it is questionable if these differences will suffice to identify unknown field isolates to the species level. In contrast, *B. vietnamiensis*, *B. glumae*, and the other species examined all appeared distinguishable.

Genome structure and implications in taxonomy. The *B. cepacia* genome exhibits a number of salient features. A remarkable variability in genome size and organization has been reported, and a considerable number of insertion sequences have been described (4, 20, 27). A comparison of the 16S rRNA sequences explained some of this variability, as one of the strains examined was shown to be a close neighbor of *B. caryophylli*, not of *B. cepacia* (20). Several other strains (including *B. cepacia* genomovars I and II and *B. vietnamiensis* strains) were reported to share more than 98% of their 16S rRNA sequences. This is obviously not a unique situation: biochemically similar groups with more than 98% 16S rRNA sequence similarity and a low level of DNA-DNA hybridization are present in many genera and are considered different species if sufficient differential phenotypic characteristics are available (29, 36, 41). However, the genomes of *B. cepacia* and related species (including *B. glumae*, *R. pickettii*, *R. eutropha*, *R. solanacearum*, and *B. glathei*) are unusual because of their organization in multiple (two to four) chromosomes or replicons. The cumulative molecular size of these individual DNA circles of presumed *B. cepacia* strains varies between 4.6 and 8.1 Mb (20). The genomic molecular sizes for genomovar I strains LMG 1222^T (=ATCC 25415^T) and LMG 2161 (=ATCC 17759) are 8.0 and 7.9 Mb, respectively, while a value of 6.8 Mb was reported for a genomovar II strain (LMG 17588 [=ATCC 17616]). At present, it has not been established that this molecular size is more or less constant within the genomovars, although this is what one would expect from a "normal" bacterial species. It is tempting to speculate on the source of this biological diversity. Could these genomovars have emerged out of each other by the loss or accumulation of large genomic fragments or even entire replicons? The present evidence derived from comparative study of DNA fragments generated by macrorestriction fragment analysis does not support this hypothesis (20), as there is a total lack of common fragments between the patterns of different strains examined. All four *B. cepacia* genomovars and *B. vietnamiensis* have the same DNA base ratio (Table 3), so it seems unlikely that some of these replicons are derived from remotely related species. Whether or not these genomovars emerged out of each other, they are composed of different isolates, mostly derived from various

sources and from different geographical areas. Whatever the underlying evolutionary events, each of the genomovars comprises several distinct strains, as determined by a variety of DNA-based typing methods (38), having a high level of overall DNA homology and a common set of proteins as do the majority of bacterial species.

Practical implications. Because of the increased interest in *B. cepacia*, several studies focused on strategies for the identification of *B. cepacia* strains. Cellular fatty acid analysis, different commercial systems based on phenotypic tests, DNA probes derived from 16S and 23S rRNA genes, and growth on selective media (17, 19, 28) were all evaluated and were often shown to be either not very sensitive or not very specific or both. From the data shown in the present study, it is obvious that a reevaluation of the specificity and sensitivity of each of these strategies is warranted.

The heterogeneity among presumed *B. cepacia* strains may have other important practical implications. Cystic fibrosis patients are particularly at risk for *B. cepacia* infections, and draconian segregation policies for *B. cepacia*-colonized patients have reduced but not entirely eliminated the incidence of colonization in many centers (12). There is also an unexplained variability in the outcome of *B. cepacia* infection in cystic fibrosis patients. Some patients succumb to *B. cepacia* syndrome (15) and die within weeks or months after colonization; others remain stable for years. Although the outcome of infection by the same genetic clone, as determined by highly discriminatory typing methods, may vary among patients, part of these differences could be explained by differences among individual *B. cepacia* strains. Closely related species may have the same pathogenic potential, and it is likely that virulence factors will be exchanged more readily between closely related taxa. However, this is not necessarily the case, as demonstrated by *B. mallei* and *B. pseudomallei*, or additionally, by *Bordetella pertussis* and *Bordetella bronchiseptica*. These taxa share a high level of DNA-DNA binding (>80%; i.e., considerably higher than the binding levels among the different *B. cepacia* genomovars) but have very different pathogenic potentials. The intercontinental Edinburgh-Toronto strain (the so-called ET12 epidemic lineage represented by strain LMG 16656 [=J2315] [11, 13, 32]) responsible for many deaths among cystic fibrosis patients belongs to genomovar III. Other epidemic strains which caused multiple deaths among cystic fibrosis patients in the United Kingdom (the Newcastle and Manchester outbreaks [28], represented by strains LMG 16658 and LMG 16659, respectively) also belong to genomovar III. However, another epidemic strain affecting mainly healthy children belongs to genomovar II (the Glasgow outbreak [42], represented by strain LMG 16660).

In the present study, the majority of "*B. cepacia*" strains isolated from cystic fibrosis patients belong to genomovar III (Table 1) (49 strains out of a total of 70 cystic fibrosis isolates) and genomovar II (12 strains), while few isolates belong to genomovars I (1 strain) and IV (5 strains) or *B. vietnamiensis* (2 strains) (one strain, LMG 14939, occupied a separate taxonomic position). It should be emphasized that the distribution of genomovars in the cystic fibrosis patient community (or other niches) has not been systematically determined and that the pathogenic significance of the different genomovars is not known.

The problem is given an extra dimension by agricultural microbiologists who continue to develop *B. cepacia* as a biocontrol agent to use its antifungal activity and its nutritional versatility for bioremediation. Clearly, the roles and pathogenic significance of *B. cepacia* genomovars, *B. vietnamiensis*, and *B. gladioli*, all of which occur in cystic fibrosis patients,

must be established in order to propose scientifically founded policies for each of the problems.

Conclusions. We performed an extensive phenotypic and genotypic analysis of 128 *Burkholderia* strains and related bacteria. Our data show that strains identified as *B. cepacia* by means of biochemical tests or cellular fatty acid analysis may belong to at least five different species. It has been proposed to refer to this group of five phenotypically similar species as the *B. cepacia* complex (third meeting of the International *Burkholderia cepacia* Working Group, May 10 to 11, 1997, Victoria, British Columbia, Canada). *B. cepacia* genomovar II, for which we propose the name *B. multivorans* below, and *B. vietnamiensis* are identifiable by means of classical phenotypic tests (Table 5), whole-cell protein electrophoresis (Fig. 1), and DNA-DNA hybridization experiments (Table 3); *B. cepacia* genomovars I, III, and IV can be differentiated by means of whole-organism protein electrophoresis (Fig. 1) and DNA-DNA hybridization experiments (Table 3). Representative strains of genomovars II (LMG 13010 [=NCTC 13007], LMG 14273, and LMG 14293), III (LMG 12614 [=NCTC 13010] [biovar c]), LMG 12615 [=NCTC 13008] [biovar a], LMG 14276 [=NCTC 13009] [biovar b], LMG 14271 [biovar status not determined], and LMG 14274 [biovar status not determined]), and IV (LMG 14294 [=NCTC 13011] and LMG 14940) have been deposited in the BCCM/LMG and NCTC culture collections.

"*Pseudomonas multivorans*" (30) was shown to represent the same taxon as *Pseudomonas cepacia*, and therefore this name lost standing in bacterial nomenclature. We chose to use this specific epithet for the *B. cepacia* genomovar II strains because it both highlights the nutritional versatility of these organisms and implicitly refers to their relatedness to *B. cepacia* strains.

Description of *Burkholderia multivorans* sp. nov. *Burkholderia multivorans* (mul.ti'vo.rans L. adj. *multus*, much; L. part. adj. *vorans*, devouring, digesting; M.L. part. adj. *multivorans* digesting many compounds) cells are 1.0 to 2.0 μ m long and 0.6 to 0.9 μ m wide and are motile at room temperature and at 37°C. Growth is observed at 37 and 42°C, not at 5°C; some strains grow at room temperature. So far, no pigmented strains have been detected and no melanin-like pigment is produced on tyrosine agar. Growth on MacConkey agar and Simmons citrate agar. No alkaline reaction in Hugh and Leifson OF medium. Oxidative in OF medium. Alkaline reaction on Christensen's citrate agar. Growth in the presence of cetrinide. No growth in the presence of 0.4% selenite. Tolerance to KCN is strain dependent. No fluorescence on King's B medium. Tyrosine, Tween 20, and Tween 80 are hydrolyzed. Urease, catalase, oxidase, beta-galactosidase (9 of 10 strains tested), and lecithinase activities are present. Nitrate is reduced, not nitrite. No indole, hydrogen sulfide, or 3-ketolactose production; no liquefaction of gelatin; no hydrolysis of esculin, casein, or starch; and no DNase activity. No lysine or ornithine decarboxylase, arginine dihydrolase, or arginine desamidase activity. No oxidation of gluconate. No production of phenylpyruvic acid. Most strains utilize malonate (9 of 10 tested). Poly- β -hydroxybutyrate is utilized and present as inclusion granules. Acid production from glucose peptone water sugar but no gas. Acid production from 10% (wt/vol) glucose and from 10% (wt/vol) lactose. Acid is produced from the following sugars in ammonium salt medium: glucose, adonitol, arabinose, cellobiose, dulcitol, glycerol, inositol, lactose, maltose, mannitol, salicin (9 of 10 strains tested), sorbitol, trehalose, xylose, fructose, and ethanol (8 of 10 strains tested), but not from raffinose, rhamnose, or sucrose.

The DNA base ratio is 68 to 69 mol%. Major fatty acid components are 14:0 (about 4.8%), 14:0 3OH (about 6.4%), 16:1 ω 7c (about 6.5%), 16:0 (about 28.9%), 17:0 cyclo (about

18.2%), 16:0 3OH (about 6.8%), 18:1 (about 11.5%), and 19:0 cyclo ω 8c (about 9.7%).

So far, *B. multivorans* strains have been primarily isolated from sputum of cystic fibrosis patients but have also been isolated from an infection in a non-cystic fibrosis patient, from the hospital environment, and from soil. Pathogenicity is unknown. The type strain is LMG 13010, which was isolated in Brussels from the sputum of a cystic fibrosis patient. Its DNA base ratio is 68 mol%, and its phenotypic characteristics are the same as those described above for the species. Characteristics differentiating *B. multivorans* from related taxa are summarized in Tables 4 and 5.

Description of *Burkholderia glathei* (Zolg and Ottow 1975) comb. nov. The description of *B. glathei* is as given in Bergey's Manual of Systematic Bacteriology (21). In addition, the prominent fatty acids of *B. glathei* strains are 14:0 (about 4.3%), 14:0 3OH (about 7.5%), 16:1 ω 7c (about 13.0%), 16:0 (about 17.5%), 17:0 cyclo (about 12.1%), 16:0 3OH (about 6.2%), and 18:1 (about 32.6%). The type strain is LMG 14190.

Description of *Burkholderia pyrrocinia* (Imananka, Kousaka, Tamura, and Arima 1965) comb. nov. The description of *B. pyrrocinia* is as given in Bergey's Manual of Systematic Bacteriology (21). In addition, the prominent fatty acids of *B. pyrrocinia* LMG 14191^T are 12:0 (4.2%), 14:0 3OH (7.3%), 16:1 ω 7c (7.3%), 16:0 (22.9%), 17:0 cyclo (about 13.5%), 16:0 2OH (4.4%), 16:0 3OH (5.8%), 18:1 (22.2%), and 19:0 cyclo ω 8c (4.7%). The type strain is LMG 14191.

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