***Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients**

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A polyphasic taxonomic study was performed on 23 strains isolated from cystic fibrosis (CF) patients in the USA. These strains were tentatively identified as *Burkholderia cepacia*, *Burkholderia vietnamiensis* and *Burkholderia* or *Ralstonia* sp. using biochemical tests and 16S rDNA-based PCR assays. Visual comparison of protein profiles indicated that they belonged to a single new group ('group 13'). The polyphasic taxonomic data showed that 18 of these strains represent a new member of the *B. cepacia* complex, referred to in this report as *B. cepacia* genomovar VI, whereas the other five strains belonged to *Burkholderia multivorans*. By means of biochemical tests, *B. cepacia* genomovar VI strains can be separated from *B. cepacia* genomovars I and III, *Burkholderia stabilis*, *B. vietnamiensis* and *Burkholderia gladioli*, but not from *B. multivorans*. Separation of *B. cepacia* genomovar VI and *B. multivorans* is possible using AFLP (amplified fragment length polymorphism) fingerprinting and DNA–DNA hybridizations. Retrospective analysis of epidemiological and genotypic data suggests that strains of *B. cepacia* genomovar VI have been involved in chronic colonization of CF patients and have been spread from person to person.

Keywords: *Burkholderia cepacia* complex, cystic fibrosis, taxonomy, genomovar

INTRODUCTION

In previous studies (Vandamme *et al.*, 1997, 2000) it has been shown that presumed *Burkholderia cepacia* strains isolated from cystic fibrosis (CF) patients and the environment belong to at least five different genomic species, referred to collectively as the *B. cepacia* complex. One of these genomic species was identified as the previously described *Burkholderia vietnamiensis* (Gillis *et al.*, 1995) and the names *Burkholderia multivorans* and *Burkholderia stabilis* have been proposed for strains classified as *B. cepacia* genomovars II and IV, respectively (Vandamme *et al.*,

1997, 2000). Only *B. cepacia* genomovar III now awaits a formal binomial species name assignment, pending the availability of differential phenotypic tests (Vandamme *et al.*, 2000). In the absence of epidemic spread, *B. cepacia* only infects a small proportion of CF patients but it has a major impact on both morbidity and mortality of infected patients; the medical and psychosocial implications of *B. cepacia* colonization and segregation of colonized and non-colonized patients as a means of infection control are enormous (LiPuma, 1998a). Accurate identification of *B. cepacia* is crucial in patient management and infection control practices, but may be problematic and misidentification is relatively common (LiPuma, 1998b).

The present study was initiated by the deposit of several strains isolated from sputa of CF patients in the USA in the *B. cepacia* Research Laboratory and Repository (University of Michigan, MI, USA). Using

Abbreviations: AFLP, amplified fragment length polymorphism; CF, cystic fibrosis.

The GenBank accession numbers for the 16S rRNA gene sequences of LMG 18941, LMG 13010^T and LMG 16656 are AF175314, AF148555 and AF148556, respectively.

biochemical characterization and 16S rDNA-based PCR assays, these strains were tentatively identified as *B. cepacia*, *B. vietnamiensis* and *Burkholderia* or *Ralstonia* sp. However, subsequent visual comparison of their protein profiles revealed that these were different and they were tentatively classified as 'group 13'. This report describes the polyphasic taxonomic study which was used for the further characterization of these isolates. The genotypic and phenotypic characteristics allowed the classification of 18 of these strains as a new member of the *B. cepacia* complex which is referred to as *B. cepacia* genomovar VI.

METHODS

Bacterial strains and growth conditions. *B. cepacia* genomovar VI, *Burkholderia pseudomallei*, *B. stabilis* and *B. multivorans* strains used in this study are listed in Table 1. Reference strains of other *Burkholderia* species and genomovars have been described in detail previously (Vandamme *et al.*, 1997). All strains were grown aerobically on Trypticase Soy Agar (BBL) and incubated at 28 °C unless otherwise indicated.

SDS-PAGE of whole-cell proteins. Strains were grown on nutrient agar (Oxoid CM3) supplemented with 0.04% (w/v) KH_2PO_4 and 0.24% (w/v) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (pH 6.8) and incubated for 48 h at 28 °C. Preparation of whole-cell proteins and SDS-PAGE were performed as described previously (Pot *et al.*, 1994). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis using the Pearson product-moment correlation coefficient were performed using the GELCOMP 4.2 software package (Applied Maths).

DNA preparation. DNA was prepared as described by Pitcher *et al.* (1989).

Amplified fragment length polymorphism (AFLP) fingerprinting. The preparation of template DNA (using restriction enzymes *ApaI* and *TaqI*), amplification [using primers A00 and T00 in the preselective PCR and primers B07 (labelled with the fluorescent dye 6-FAM) and T11 in the selective PCR], separation of the fragments using an ABI Prism 377 automated DNA sequencer and numerical analysis were performed as described previously (Coenye *et al.*, 1999b).

16S rDNA sequencing. The nearly complete sequence (corresponding to positions 8 to 1541 in the *Escherichia coli* numbering system) of the 16S rRNA gene of strains LMG 13010^T, LMG 16656 and LMG 18941 was amplified by PCR using conserved primers [5'-AGAGTTTGATCCTGGC-TCAG-3' (5'-CTGGCTCAGGAC/TGAACGCTG-3' for LMG 18941) and 5'-AAGGAGGTGATCCAGCCGCA-3']. The PCR product was purified using a QIAquick PCR Purification kit (Qiagen) according to the manufacturer's instructions. Sequence analysis was performed using an Applied Biosystems 377 DNA sequencer and the protocols of the manufacturer (Perkin Elmer) using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit. The sequencing primers are those given by Coenye *et al.* (1999a). Sequence assembly was performed by using the program AUTOASSEMBLER (Perkin Elmer). A phylogenetic tree was constructed with the GENCOMP 2.1 software package (Applied Maths), based on the neighbour-joining method

(Saitou & Nei, 1987). Approximately 1460 bases were used and all unknown bases were excluded for the calculations.

Determination of the DNA base composition. DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The obtained nucleoside mixture was then separated by HPLC using a Waters SymmetryShield C8 column thermostatted at 37 °C. The solvent was 0.02 M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 4.0) with 1.5% acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference.

DNA-DNA hybridizations. DNA-DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989) using a HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. The hybridization temperature was 50 °C.

rRNA-based PCR assay. Assays for the identification of members of the *B. cepacia* complex based on the rRNA operon are described in detail in a separate study (LiPuma *et al.*, 1999). The following primers were used: RHG-F and RHG-R (specific for all of the *Burkholderia* and *Ralstonia* species); PC-SSF and PC-SSR (specific for *B. cepacia* genomovars I and III, and *B. stabilis*); BC-GII and BC-R (specific for *B. multivorans*); and BC-V and BC-R (specific for *B. multivorans* and *B. vietnamiensis*).

Fatty acid methyl ester analysis. After an incubation period of 24 h at 35 °C, a loopful of well-grown cells was harvested and fatty acid methyl esters were prepared, separated and identified using the Microbial Identification System (Microbial ID) as described previously (Vandamme *et al.*, 1992).

Phenotypic characterization. Tests were performed as previously described (Henry *et al.*, 1997). Briefly, pure cultures were stored at -70 °C in Mueller-Hinton broth (Difco) with 8% DMSO. Frozen isolates were subcultured to Columbia agar containing 5% sheep blood (PML Microbiologicals) before testing. The primary identification system used was the API Rapid NE system (bioMérieux Vitek) with glucose, maltose, lactose, xylose, sucrose and adonitol oxidation-fermentation sugars (Hugh & Leifson, 1953), and an adaptation of Moeller lysine and ornithine decarboxylases (Difco). In brief, the 2 ml test volume was overlaid with 0.5 ml light mineral oil and a heavy, visible inoculum was used. A tube without amino acid was included as a negative control. Oxidation-fermentation sugars were incubated for up to 7 d and other tests for 2 d. All incubations were done at 35 °C except where otherwise mentioned. Growth on Trypticase Soy Agar (Becton Dickinson) at 35 and 42 °C was observed for appearance and pigment. Phytopathogenicity was tested as described by Gonzalez & Vidaver (1979). In brief, strains were grown for 24 h at 28 °C. Bacterial suspensions of 10^9 c.f.u. ml^{-1} were made in sterile distilled water and 100 μl suspension was inoculated on 3–5 mm thick onion slices. The inoculated onion slices were incubated for 48 h at 28 °C. *B. cepacia* genomovar I strain LMG 1222^T was used as a positive control. Sterile distilled water was used as a negative control. Growth on agar selective for *B. cepacia* (DM253; Mast Diagnostics) containing ticarcillin (100 $\mu\text{g ml}^{-1}$) and polymyxin B (300 U ml^{-1}) was tested by incubating the strains at 37 °C for 48 h. Ability to grow on BCSA agar (Henry *et al.*, 1997) containing gentamicin (10 $\mu\text{g ml}^{-1}$), polymyxin (600 U ml^{-1}) and vancomycin (2.5 $\mu\text{g ml}^{-1}$) was tested by incubation at 35 °C under aerobic conditions.

Table 1 List of strains studied

Abbreviations: CCUG, Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie Gent, Universiteit Gent; ^T, type strain.

Species and strain designation	Original strain designation	Source
<i>Burkholderia cepacia</i> genomovar VI		
LMG 18941	HI2112	CF patient (USA)
R-711	HI2110	CF patient (USA)
R-726	PC543	CF patient (USA)
R-733	CEP021	CF patient (USA)
LMG 18946	AU0018	CF patient (USA)
R-2879	AU0090	CF patient (USA)
LMG 18944	PC688	CF patient (USA)
R-3074	PC560	CF patient (USA)
R-3200	AU0158	CF patient (USA)
LMG 18942	PC534	CF patient (USA)
R-4055	AU0266	CF patient (USA)
R-4225	PC520	CF patient (USA)
R-4232	AU0169	CF patient (USA)
LMG 18943	AU0645	CF patient (USA)
R-6140	AU0746	CF patient (USA)
R-6342	AU0869	CF patient (USA)
R-6918	AU0938	CF patient (USA)
R-7394	AU0981	CF patient (USA)
<i>Burkholderia stabilis</i>		
LMG 13017	88/190	Blood culture (Belgium, 1988)
LMG 14086	315/70	Respirator (UK, 1970)
LMG 14294	E3P	CF patient (Belgium, 1993)
LMG 7000	CCUG 13348	Blood (Sweden, 1983)
LMG 14295	E4P	CF patient (Belgium, 1993)
<i>Burkholderia multivorans</i>		
LMG 13010 ^T	CEPA002 ^T	CF patient (Belgium, 1992)
LMG 18824	FC147	Chronic granulomatous disease patient (Canada)
LMG 18822	C5393	CF patient (Canada)
LMG 18825	CF-A1-1	CF patient (UK)
LMG 17588	CEP144	Soil (USA)
R-2453	AU0064	CF patient (USA)
R-2454	AU0066	CF patient (USA)
R-4543	96/47	CF patient (UK)
LMG 18945	97/35	CF patient (UK)
R-4528	HI2249	CF patient (USA)
LMG 14273	A5P	CF patient (Belgium, 1993)
<i>Burkholderia pseudomallei</i>		
R-4038	CCUG 218	Sheep (Australia, before 1949)
R-4039	CCUG 13790	Pus (Thailand, 1983)
R-4040	CCUG 15646	Ovine spinal abscess (Australia, 1966)

RESULTS

AFLP fingerprinting

Reproducibility was checked by preparing PCR products in duplicate and was always higher than 92 %. Following numerical analysis, 10 clusters could be delineated, while *Burkholderia pyrrocinia* LMG 14191^T occupied a separate position (Fig. 1). The *B.*

cepacia genomovar VI strains investigated appeared as a homogeneous group which formed cluster I. *Burkholderia gladioli*, *Burkholderia plantarii* and *Burkholderia glumae* reference strains constituted clusters II, III and VIII, respectively. Reference strains of *B. vietnamiensis* formed cluster IV. Cluster V comprised three *B. cepacia* genomovar III reference strains. Representatives of *B. stabilis* and *B. multi-*

Fig. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients between the AFLP patterns of the strains studied. The correlation coefficient is expressed as percentage similarity for convenience.

Fig. 2. Electrophoretic protein patterns of a selection of strains investigated. The molecular mass markers used (lane at the bottom) were (from left to right): lysozyme (14.5 kDa); trypsin inhibitor (20 kDa); trypsinogen (24 kDa); carbonic anhydrase (29 kDa); glyceraldehyde-3-phosphate dehydrogenase (36 kDa); egg albumin (45 kDa); bovine albumin (60 kDa); and β -galactosidase (116 kDa).

vorans formed clusters VI and VII, respectively. Cluster IX was composed of three *Burkholderia pseudomallei* strains. Finally, three *B. cepacia* genomovar I strains, including the type strain, constituted cluster X.

SDS-PAGE of whole-cell proteins

Reproducibility was checked by preparing protein extracts in duplicate. The correlation level between the patterns obtained with different extracts of the same strain was more than 93% (data not shown). Following numerical analysis, 'group 13' strains formed two separate clusters. A first cluster contained 12 *B. cepacia* genomovar VI strains (R-3074, R-733, R-711, LMG 18941, LMG 18942, R-4225, LMG 18944, R-6342, LMG 18943, R-6918, R-3200 and R-726). A second cluster contained *B. cepacia* genomovar VI strains R-4232, R-2879, R-4055, R-7394, LMG 18946 and R-6140, and *B. multivorans* R-4528, R-4543 and LMG 18945. Two 'group 13' strains (R-2453 and R-2454) grouped together with *B. multivorans* reference strains (data not shown). A selection of protein patterns of representative strains of the *B. cepacia* complex and *B. gladioli* is shown in Fig. 2.

16S rRNA gene sequencing

The 16S rRNA gene sequence of strain LMG 18941 (AF175314) was compared with available 16S rRNA gene sequences of representatives of the β -Proteobacteria and with the 16S rDNA gene sequences of *B. multivorans* LMG 13010^T (AF148555) and *B. cepacia* genomovar III LMG 16656 (AF148556) (Fig. 3). LMG 18941 was closely related to members of the *B. cepacia* complex, with similarity levels ranging from 98.3 to 99.3%. Similarity levels towards other *Burkholderia*

species were between 98.1 and 94.8%. Similarity levels towards representatives of other taxa belonging to the β -Proteobacteria were below 91.0%.

DNA–DNA hybridizations and determination of the G+C content

Hybridization results revealed that DNA–DNA binding values between *B. cepacia* genomovar VI LMG 18941 and (i) *B. cepacia* genomovar I LMG 1222^T (53%), (ii) *B. cepacia* genomovar III LMG 16656 (37%), (iii) *B. stabilis* LMG 7000 (44%) and (iv) *B. vietnamiensis* LMG 10929^T (53%) were intermediate or low. DNA–DNA binding levels of *B. cepacia* genomovar VI strains towards representatives of *B. multivorans* were between 47 and 55% (Table 2). DNA–DNA hybridization experiments carried out between *B. cepacia* genomovar VI strains LMG 18941, LMG 18942 and LMG 18943 revealed binding levels between 97 and 100%. All *B. cepacia* genomovar VI strains investigated had G+C contents between 66.9 and 67.7 mol% (Table 2).

Cellular fatty acid analysis

The mean fatty acid composition of *B. cepacia* genomovar VI strains is shown in Table 3. The predominant fatty acids were 16:0, 17:0 cyclo, 19:0 cyclo ω 8c, summed feature 3 (comprising 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with equivalent chain-length of 10.928, or 12:0 ALDE, or any combination of these fatty acids) and summed feature 7 (comprising 18:1 ω 7c, 18:1 ω 9t or 18:1 ω 12t, or any combination of these fatty acids) (summed features comprise fatty acids that can not be resolved by the Microbial Identification System). Summed feature 3 and summed

Fig. 3. Neighbour-joining phylogenetic tree showing the position of *B. cepacia* genomovar VI within the β -Proteobacteria based on 16S rDNA sequence comparisons. Accession numbers are given in parentheses. *E. coli* was used as an outgroup. Bar, 10% sequence dissimilarity.

Table 2 DNA–DNA binding values and G + C content (mol%) of all strains examined

Species	G + C (mol %)	DNA binding values with strain:					
		LMG 18941	LMG 18942	LMG 18943	LMG 13010 ^T	R-2453	LMG 18945
<i>B. cepacia</i> genomovar VI							
LMG 18941	67.4	100					
LMG 18942	66.9	100	100				
LMG 18943	67.7	100	97	100			
<i>B. multivorans</i>							
LMG 13010 ^T	67.7	53	50	48	100		
R-2453	67.4	55		48	87	100	
LMG 18945	66.9			47	85	91	100

feature 7 probably correspond to 14:0 3-OH and 18:1 ω 7c, respectively, as these fatty acids have been reported in *Burkholderia* species (Stead, 1992).

rRNA-based PCR assay

The 16S rRNA gene of all *B. cepacia* genomovar VI strains investigated was amplified with primer pair RHG-F and RHG-R, whereas no amplification prod-

uct was obtained using primer pair PC-SSF and PC-SSR, indicating that the strains belonged to the genera *Burkholderia* or *Ralstonia*, but were different from *B. cepacia* genomovars I and III and from *B. stabilis*. Using the *B. multivorans*-specific primer pair BC-GII and BC-R, an amplicon of 435 bp was obtained for strain R-4232 only. Primer pair BC-V and BC-R (specific for *B. multivorans* and *B. vietnamiensis*) amplified a 848 bp fragment of the 16S rRNA gene of

Table 3 Fatty acid composition of the strains studied

Composition is expressed as percentage. Those fatty acids for which the mean amount for all taxa was less than 1% are not given. Mean percentages and standard deviations are given. The number of strains containing the fatty acid is given in parentheses only when not present in all of the strains examined. Tr, trace amount (less than 1%).

Strain	14:0	16:0	17:0 cyclo	16:1 2-OH	16:0 2-OH	16:0 3-OH	18:0	19:0 cyclo ω 8c	18:1 2-OH	Summed feature 3*	Summed feature 4*	Summed feature 7*
<i>B. cepacia</i> genomovar I (14 strains)†	3.7±1.6	26.8±2.8	17.9±3.6	Tr (11)	3.3±0.9	6.6±0.9	Tr (13)	12.5±4.7	3.4±0.8	6.0±1.4	4.6±2.5	11.8±3.5
<i>B. cepacia</i> genomovar III (48 strains)†	4.2±0.6	26.1±3.5	21.3±4.2	1.3±0.6 (38)	3.2±0.9	6.7±1.5	2.4±2.2 (45)	13.4±4.9	2.3±1.0 (41)	6.3±1.5	3.0±1.0	9.3±4.4
<i>B. stabilis</i> (11 strains)†	4.4±0.5	25.6±4.3	17.8±3.3	Tr (8)	4.5±1.5	5.7±0.9	Tr (8)	15.3±4.2	2.4±0.6	5.1±0.5	2.7±0.8	10.7±1.8
<i>B. vietnamiensis</i> (4 strains)†	3.8±0.6	19.5±2.5	14.0±4.9	1.3±0.2	2.8±0.4	6.4±0.7	5.2±3.4	5.8±2.5	3.4±0.5	6.3±0.9	9.8±2.5	19.7±5.5
<i>B. multivorans</i> (14 strains)†	4.8±0.6	28.9±3.5	18.2±4.9	1.3±0.8 (12)	2.8±1.0	6.8±0.5	Tr (5)	9.7±3.3	1.3±0.7 (12)	6.4±1.1	6.5±1.5	11.5±4.8
<i>B. gladioli</i> (4 strains)†	4.9±0.2	29.0±1.1	17.2±1.9	1.5±0.2	3.2±0.3	7.1±1.0	Tr	10.0±2.7	3.5±0.7	6.8±1.1	5.1±2.3	11.0±3.7
<i>B. cepacia</i> genomovar VI (11 strains)	4.2±0.4	26.1±1.4	16.0±2.2	1.3±0.4	3.3±1.0	6.2±0.6	Tr	14.8±3.7	1.6±0.6	8.7±1.1	4.2±2.0	9.9±4.3

* Summed feature 3 comprises 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with equivalent chain length value of 10.928, or 12:0 ALDE, or any combination of these fatty acids. Summed feature 4 comprises 16:1 ω 7c or 15 iso 2-OH or both. Summed feature 7 comprises 18:1 ω 7c, 18:1 ω 9t, 18:1 ω 12t, or any combination of these fatty acids.

† Data from Vandamme *et al.* (1997, 2000).

strains R-6918, R-6140 and R-7394, but not from the other *B. cepacia* genomovar VI strains.

Phenotypic characterization

None of the strains tested was able to cause soft rot of onions under the conditions used. Both *B. cepacia* selective media supported growth of all strains investigated. The following features were present in all strains investigated: oxidase and β -galactosidase activity, denitrification, growth at 42 °C, acidification of glucose, lactose, maltose, xylose and adonitol and assimilation of glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. The following features were absent in all strains investigated: lysine and ornithine decarboxylase, tryptophanase, arginine dihydrolase and urease activity, aesculin hydrolysis, assimilation of maltose, gelatin liquefaction, haemolysis and pigment production.

DISCUSSION

A polyphasic taxonomic study was performed to characterize 23 strains isolated from CF patients in the United States. Preliminary analysis using the API Rapid NE system identified these strains as members of the *B. cepacia* complex and this identification was confirmed by key positive biochemical reactions as described previously (Henry *et al.*, 1997). The results of the present study allowed 18 of these strains to be identified as new members of the *B. cepacia* complex, *B. cepacia* genomovar VI.

Characterization of *B. cepacia* genomovar VI

AFLP, a genomic fingerprinting technique based on the selective amplification and visualization of restriction fragments from a total digest of genomic DNA, allowed differentiation of *Burkholderia* species

and genomovars and its results are generally consistent with DNA–DNA hybridization levels (Coenye *et al.*, 1999b). It was also shown that the AFLP procedure is a valuable or even superior alternative for SDS-PAGE of whole-cell proteins to differentiate *Burkholderia* species (Coenye *et al.*, 1999b). In this study, all *B. cepacia* genomovar VI strains investigated formed a single homogeneous cluster and could easily be differentiated from other *Burkholderia* species and genomovars (Fig. 1).

It has been reported that SDS-PAGE of whole-cell proteins is a good screening method for the differentiation of members of the *B. cepacia* complex (Vandamme *et al.*, 1997). However, subsequent studies have shown that, using this method, several *B. cepacia* genomovar I and *B. stabilis* strains were misidentified (Coenye *et al.*, 1999b; Vandamme *et al.*, 2000). In the present study, it was shown that the majority of 'group 13' strains were identified correctly as *B. cepacia* genomovar VI, following numerical analysis and visual comparison of the protein profiles; but several *B. multivorans* strains were also identified as *B. cepacia* genomovar VI.

Comparison of the 16S rDNA sequence of strain LMG 18941 with the available sequences of other *Burkholderia* species indicated that its closest relatives are members of the *B. cepacia* complex (similarity levels ranging from 98.3 to 99.3%). The DNA base ratio of 66.9–67.7 mol% is consistent with that of the other members of the *B. cepacia* complex (67–69 mol%; Vandamme *et al.*, 1997). However, DNA–DNA hybridizations between LMG 18941 and representative strains of the other members of the *B. cepacia* complex revealed only low or intermediate binding values, whereas DNA–DNA binding values towards strains belonging to the same AFLP cluster (LMG 18942 and LMG 18943) were high. These data unambiguously indicate that this strain represents a novel taxon within the *B. cepacia* complex.

Table 4 Phenotypic characteristics useful for the differentiation of *B. cepacia* genomovar VI from other members of the *B. cepacia* complex and *B. gladioli*

Strains: 1, *B. cepacia* genomovar VI (14 strains); 2, *B. multivorans* (10 strains); 3, *B. cepacia* genomovar I (11 strains); 4, *B. cepacia* genomovar III (39 strains); 5, *B. stabilis* (5 strains); 6, *B. vietnamiensis* (7 strains); 7, *B. gladioli* (12 strains). +, All strains give a positive reaction, –, all strains give a negative reaction, v, strain dependent result; all data except for *B. cepacia* genomovar VI from Vandamme *et al.* (1997, 2000) and own unpublished results.

Test	1	2	3	4	5	6	7
Growth at 42 °C	+	+	–	+	–	–	–
β-Galactosidase activity	+	+	+	+	–	+	+
Decarboxylation of:							
Lysine	–	v	+	+	+	+	+
Ornithine	–	–	+	+	+	+	+
Oxidation of:							
Sucrose	–	–	+	+	–	+	–
Lactose	+	+	+	v	+	+	–
Adonitol	+	+	+	v	+	+	–
Maltose	+	+	+	v	+	+	–

Identification of *B. cepacia* genomovar VI

Biochemically, *B. cepacia* genomovar VI cannot be distinguished from *B. multivorans*, but it can be distinguished from the other members of the *B. cepacia* complex and from *B. gladioli*. The most important differential biochemical tests are summarized in Table 4.

As can be seen in Table 3, the mean fatty acid profile of *B. cepacia* genomovar VI is virtually indistinguishable from that of the other members of the *B. cepacia* complex and from *B. gladioli*, although differentiation from *B. vietnamiensis* is possible based on the higher amounts of 16:0 and 19:0 cyclo ω 8c and the lower amounts of 18:0, summed feature 4 and summed feature 7 present in *B. cepacia* genomovar VI.

Recently, an rRNA-based PCR assay for the identification of *B. cepacia* complex isolates was described (LiPuma *et al.*, 1999). Most *B. cepacia* genomovar VI strains did not react with any of the species-specific primers designed against the rRNA operon. One strain (R-4232), however, reacted positively with the primers specific for *B. multivorans* and three strains (R-6918, R-6140 and R-7394) reacted positively with the primers designed for *B. vietnamiensis*. It was reported that the specificity of these primers was 92 and 99% for *B. vietnamiensis* and *B. multivorans*, respectively (LiPuma *et al.*, 1999). Our results indicate that the specificity of the *B. vietnamiensis*-specific primers will be lower than reported since, as well as some *B. multivorans* strains (LiPuma *et al.*, 1999), *B. cepacia* genomovar VI strains can give rise to false-positive results. Despite these shortcomings, this PCR assay is a useful and easy tool for the separation of *B. cepacia*

genomovar VI from *B. cepacia* genomovars I and III and from *B. stabilis*.

Conclusion

The results of our genotypic and phenotypic analysis show that these strains belong to a new genomovar within the *B. cepacia* complex. This new genomovar can be identified by AFLP fingerprinting and DNA–DNA hybridizations and can be separated from *B. cepacia* genomovars I and III, *B. stabilis*, *B. vietnamiensis* and *B. gladioli*, but not from *B. multivorans* by phenotypic tests and SDS-PAGE of whole-cell proteins.

According to the guidelines proposed by Ursing *et al.* (1995), this new genomic species should be named *B. multivorans* genomovar II, pending the availability of differential diagnostic tests (Wayne *et al.*, 1987; Ursing *et al.*, 1995). However, since *B. multivorans* was originally known as *B. cepacia* genomovar II, the name *B. multivorans* genomovar II would cause significant confusion. In addition, several strains were initially identified as *B. vietnamiensis* by species-specific PCR. Given the close relationship of this new organism with the other members of the *B. cepacia* complex, it is proposed that they are referred to as *B. cepacia* genomovar VI.

Retrospective analysis of epidemiological and genotypic data suggests that strains of *B. cepacia* genomovar VI can chronically colonize CF patients and may spread from person to person (LiPuma *et al.*, 1990, 1994). Additional studies are needed to ascertain the clinical implications of respiratory tract colonization with this genomovar. *B. cepacia* genomovar VI strains LMG 18941, LMG 18942, LMG 18943, LMG 18944 and LMG 18946 have been deposited in the BCCM/LMG Bacteria Collection (University of Gent, Gent, Belgium).

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