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Burkholderia fungorum sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples

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A polyphasic taxonomic study that included DNA–DNA hybridizations, DNA base ratio determinations, 16S rDNA sequence analyses, whole-cell protein and fatty acid analyses and an extensive biochemical characterization was performed on 16 strains isolated from the environment, animals and human clinical samples. The isolates belonged to the genus *Burkholderia*, were phylogenetically closely related to *Burkholderia graminis*, *Burkholderia caribensis* and *Burkholderia phenazinium* and had G+C contents between 61·9 and 62·2 mol%. Seven strains isolated from the rhizosphere were assigned to *Burkholderia caledonica* sp. nov. [type strain LMG 19076^T (= CCUG 42236^T)]. Nine strains isolated from the environment, animals and human clinical samples were assigned to *Burkholderia fungorum* sp. nov. [type strain LMG 16225^T (= CCUG 31961^T)]. Differential tests for *B. graminis*, *B. caribensis*, *B. phenazinium*, *B. caledonica* and *B. fungorum* include the following: assimilation of trehalose, citrate, DL-norleucine, adipate and sucrose; nitrate reduction; growth in the presence of 0·5% NaCl; and β-galactosidase activity.

Keywords: *Burkholderia fungorum*, *Burkholderia caledonica*, *Burkholderia graminis*, *Burkholderia caribensis*, taxonomy

INTRODUCTION

The genus *Burkholderia* currently comprises 19 named species: *Burkholderia andropogonis*, *Burkholderia caribensis*, *Burkholderia caryophylli*, *Burkholderia cepacia* (comprising genomovars I, III and VI), *Burkholderia gladioli*, *Burkholderia glathei*, *Burkholderia glumae*, *Burkholderia graminis*, *Burkholderia kururiensis*, *Burkholderia mallei*, *Burkholderia multivorans*, *Burkholderia phenazinium*, *Burkholderia plantarii*, *Burkholderia pseudomallei*, *Burkholderia pyrrocinia*, *Burkholderia stabilis* (formerly *B. cepacia* genomovar IV), *Burkholderia thailandensis*, *Burkholderia ubonensis* and *Burkholderia vietnamiensis* (Yabuuchi *et al.*, 1992, 1995, 2000; Gillis *et al.*, 1995; Vandamme *et al.*, 1997, 2000; Viallard *et al.*, 1998; Brett *et al.*, 1998; Achouak *et al.*, 1999; Coenye *et al.*, 1999a, 2001; Zhang *et al.*, 2000). Within this genus, there are several stable phylogenetic clusters, one of which contains *B. graminis* (an organism isolated from agricultural soils in France and Australia), *B. caribensis* (an exopolysaccharide-producing organism isolated from the soil in Martinique), *B. phenazinium*, *B. glathei* (both isolated from soil) and several unidentified xenobiotic-compound-degrading strains (Viallard *et al.*, 1998; Achouak *et al.*, 1999).

In an ongoing survey of *B. cepacia*-like organisms (using whole-cell protein analysis), 16 isolates from human, animal and environmental specimens exhibited striking similarity to *B. graminis* and *B. caribensis* strains. This prompted the polyphasic taxonomic study described below.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains LMG 16225^T (= CCUG 31961^T), LMG 16307 and LMG 19076^T (= CCUG 42236^T) described in this work are respectively AF215705, AF215706 and AF215704.

METHODS

Bacterial strains and growth conditions. Reference strains of *B. graminis*, *B. caribensis*, *B. phenazinium* and *B. glathei* and the 16 novel *B. cepacia*-like isolates are listed in Table 1. Reference strains of *B. andropogonis*, *B. caryophylli*, *B. cepacia* genomovars I, III and VI, *B. gladioli*, *B. glumae*, *B. multivorans*, *B. plantarii*, *B. pseudomallei*, *B. pyrrocinia*, *B. stabilis* and *B. vietnamiensis* have been described previously (Vandamme *et al*., 1997, 2000; Coenye *et al*., 2001). The seven strains here assigned to *Burkholderia caledonica* sp. nov. were isolated from rhizosphere soil, as described previously (Butler *et al*., 1995). Strains were grown aerobically on Trypticase Soy Agar (BBL) and incubated at 37 °C unless otherwise indicated.

SDS-PAGE of whole-cell proteins. Strains were grown on nutrient agar (CM3; Oxoid) supplemented with 0·04 % (w/v) KH_2PO_4 and 0·24 % (w/v) $Na_2HPO_4.12H_2O$ (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 Pearson's product-moment correlation coefficient were performed using the GELCOMPAR 4.2 software package (Applied Maths).

Table 1. List of *Burkholderia* strains studied

Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; NCIB, National Collection of Industrial Bacteria, Aberdeen, UK.

Original strain designation	BCCM/LMG strain no.	Other strain designation	Origin	Source
B. caribensis				
MWAP64^T	LMG 18531^T	CCUG 42847^T	T. Heulin	Vertisol (Martinique)
MWAP84	LMG 18532	CCUG 42848	T. Heulin	Vertisol (Martinique)
B. glathei				
ATCC 29195^T	LMG 14190^T	–	ATCC	Lateritic soil (Germany)
ATCC 29198	LMG 14932	–	ATCC	Lateritic soil (Germany)
B. graminis				
ATCC 700544^T	LMG 18924^T	CCUG 42231^T	ATCC	Maize senescent root system (France)
C3A1M	LMG 18947	CCUG 42844	J. Balandreau	Maize senescent root system (France)
C5A1M	LMG 18948	CCUG 42845	J. Balandreau	Maize senescent root system (France)
AUS33	LMG 18949	CCUG 42843	J. Balandreau	Wheat pasture rotation (Kapunda, Australia)
B. phenazinium				
NCIB 11027^T	LMG 2247^T	CCUG 20836^T	NCIB	Soil enriched with threonine
NCIB 11431	LMG 6868	CCUG 42269	NCIB	
***B. fungorum* sp. nov.**				
Croize P763-2^T	LMG 16225^T	CCUG 31961^T	F. Seigle-Murandi	*Phanerochaete chrysosporium*
BIOLOG 1786	LMG 18809	CCUG 22618	B. Bochner	
LMG 18810	LMG 18810	CCUG 30784	K. Eriksson	Haemoglobin solution (Sweden, 1992)
Croize PK32	R-3471	CCUG 31959	F. Seigle-Murandi	*Phanerochaete chrysosporium*
V02 10158	R-8655	CCUG 42846	R. Zbinden	Vaginal secretion of pregnant woman
SVA B1549/99	R-9502	CCUG 42397	E. Eriksson	Mouse nose (1999)
SVA B1555/99	R-9503	CCUG 42398	E. Eriksson	Mouse nose (1999)
GDXE P1196(1)	LMG 16226	CCUG 32541	F. Seigle-Murandi	*Phanerochaete chrysosporium*
LMG 16307	LMG 16307	CCUG 18424	PHLS Sundsvall, Sweden	Cerebrospinal fluid of 66-year-old woman (Sweden, 1986)
***B. caledonica* sp. nov.**				
W50D^T	LMG 19076^T	CCUG 42236^T	Our isolate	Rhizosphere soil (Edinburgh, UK)
W51E	LMG 19077	CCUG 42237	Our isolate	Rhizosphere soil (Edinburgh, UK)
W53B	LMG 19078	CCUG 42238	Our isolate	Rhizosphere soil of vine plant (Edinburgh, UK)
W73C	R-4186	CCUG 42239	Our isolate	Sandy rhizosphere soil (Abernethy Forest, UK)
W47F	R-4187	CCUG 42233	Our isolate	Rhizosphere soil (Edinburgh, UK)
W50C	R-4188	CCUG 42235	Our isolate	Rhizosphere soil (Edinburgh, UK)
W49D	R-4192	CCUG 42234	Our isolate	Rhizosphere soil (Edinburgh, UK)

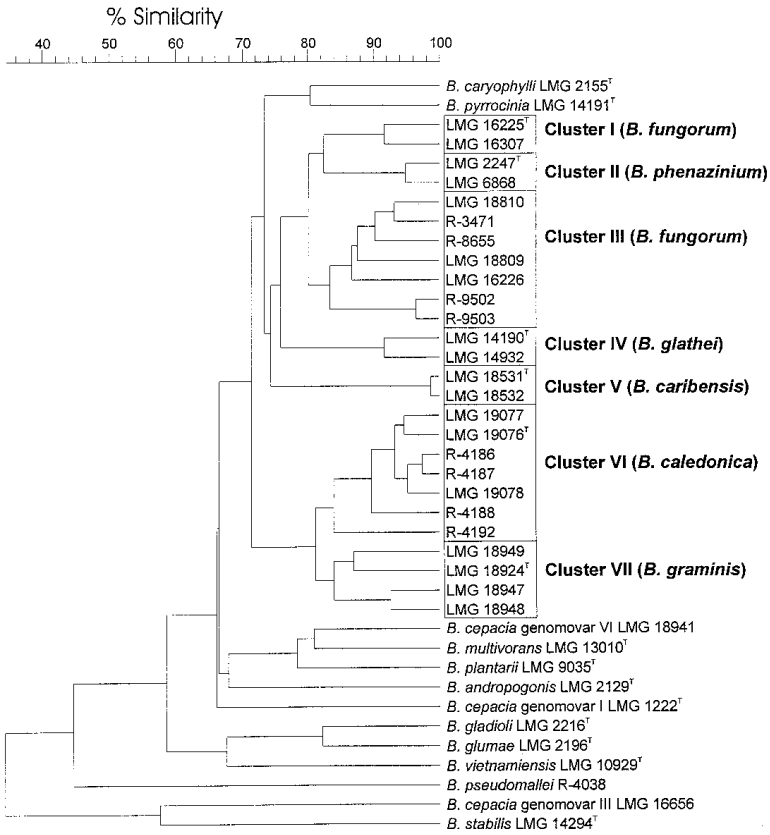

Fig. 1. Dendrogram derived from the unweighted pair group mean linkage of correlation coefficients between the protein patterns of the strains studied. For convenience, the correlation coefficients are expressed as percentages of similarity.

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

16S rDNA sequencing. The nearly complete sequences (corresponding to positions 8–1541 in the *Escherichia coli* numbering system) of the 16S rRNA genes of strains LMG 16225^T (= CCUG 31961^T), LMG 16307 and LMG 19076^T (= CCUG 42236^T) were amplified by the PCR using conserved primers 5′-AGAGTTTGATCCTGGCTGAG-3′ (positions 8–27) and 5′-AAGGAGGTGATCCAGCCGCA-3′ (positions 1521–1541) (Coenye *et al.*, 1999b). The PCR products were purified using a QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. Sequence analysis was performed using an Applied Biosystems 310 DNA sequencer and the protocols of the manufacturer (Perkin-Elmer), with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit. The sequencing primers were those given by Coenye *et al.* (1999b). Sequence assembly was performed by using the program AUTOASSEMBLER (Perkin-Elmer). Phylogenetic analysis was performed using the GENECOMPAR 2.1 software package (Applied Maths). A phylogenetic tree based on the neighbour-joining method was constructed.

Determination of the DNA base composition. DNA was degraded enzymically into nucleosides, as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC, using a Waters SymmetryShield C8 column with a thermostat set at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5% acetonitrile. Non-methylated λ 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 an HTS7000 Bio Assay Reader (Perkin-Elmer) for the fluorescence measurements. The hybridization temperature was 50 °C in 50% formamide. Each value given is the mean of at least two hybridization experiments.

Fatty acid methyl ester analysis. After an incubation period of 24 h at 28 °C, 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) described elsewhere (Vandamme *et al.*, 1992).

Phenotypic characterization. Classical phenotypic tests were performed as described previously (Vandamme *et al.*, 1993). API ZYM and API20 NE tests were performed according to the recommendations of the manufacturer (bioMérieux).

RESULTS

SDS-PAGE of whole-cell proteins

Whole-cell protein extracts were prepared from all 16 novel isolates and from reference strains of known *Burkholderia* species. The 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). After numerical analysis and visual comparison of the profiles, seven clusters could be delineated (Fig. 1). *B. phenazinium*, *B. glathei*, *B. caribensis* and *B. graminis* reference strains each formed distinct clusters (clusters II, IV, V and VII, respectively). Reference strains from other *Burk-*

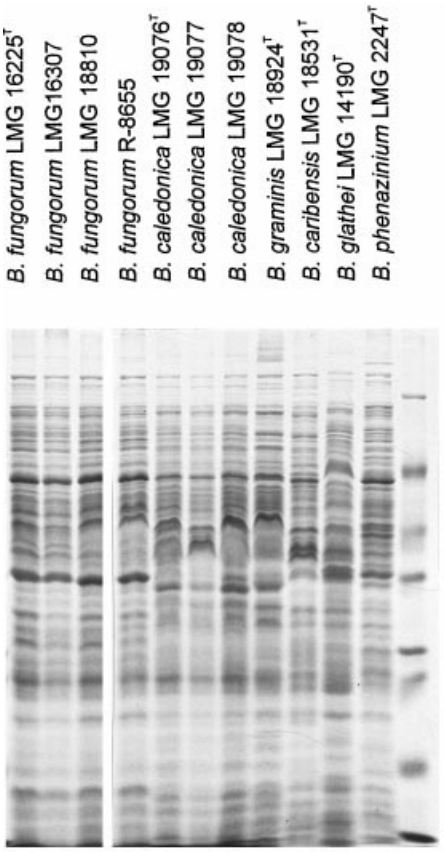

Fig. 2. Electrophoretic protein patterns of a selection of strains investigated. The M_r markers used (see right-hand lane) were (from bottom to top) lysozyme (14500), trypsin inhibitor (20000), trypsinogen (24000), glyceraldehyde-3-phosphate dehydrogenase (36000), egg albumin (45000), bovine albumin (60000) and β-galactosidase (116000).

holderia species occupied distinct positions in the dendrogram. The 16 novel isolates were distributed over three different clusters (clusters I, III and V). Cluster I was composed of an environmental isolate and a human isolate. Cluster III comprised seven strains (two from animals, two from the environment, one human isolate, one nosocomial isolate and one isolate of unknown origin). Cluster VI was composed of seven rhizosphere isolates. A selection of protein patterns of strains from each cluster is shown in Fig. 2.

16S rRNA gene sequencing

The 16S rDNA sequences of strains LMG 16225^T, LMG 16307 and LMG 19076^T were determined and compared with available 16S rDNA sequences of other representatives of the *β-Proteobacteria* and with each other. The 16S rDNA sequences of strains LMG 16225^T and LMG 16307 were identical and were closely related to those of LMG 19076^T (97·8% similarity), *B. phenazinium* LMG 2247^T (98·5% similarity), *B. graminis* LMG 18924^T (97·7% similarity) and *B. caribensis* LMG 18531^T (96·7% similarity). The similarity levels of LMG 19076^T towards *B. graminis* LMG 18924^T, *B. phenazinium* LMG 2247^T and *B. caribensis* LMG 18531^T were respectively 97·7, 97·0 and 96·7% (Fig. 3). Similarity levels towards representatives of other known *Burkholderia* species were below 95·7%. Similarity levels towards other taxa belonging to the *β-Proteobacteria* were below 95·4%.

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

DNA was prepared from strains representing the three protein-electrophoretic clusters amongst the 16 isolates and from reference strains of *B. graminis*, *B. caribensis* and *B. phenazinium* (their closest phylogenetic neighbours). The DNA–DNA binding values and the G+C contents of all strains examined are shown in Table 2. All strains investigated had G+C contents between 61·9 and 63·2 mol%. The hybridization experiments revealed that strains belonging to protein-electrophoretic cluster VI formed one genomic group (a DNA–DNA binding value of 100% was calculated between two randomly chosen isolates), whereas protein-electrophoretic clusters I and III formed a second genomic group with internal DNA–DNA binding values of between 85 and 100%. The *B. graminis* and *B. caribensis* reference strains formed a single group, each having internal DNA–DNA binding levels above 95%. The DNA binding values between representatives of the two genomic groups and between representatives of both genomic groups and reference strains of *B. caribensis* and *B. phenazinium* were below 30 and 28%, respectively. Intermediate DNA–DNA binding levels were calculated between *B. graminis* reference strains and representatives of the two genomic groups (31–48% and 32–41%, respectively).

Cellular fatty acid analysis

The cellular fatty acid composition of all *B. graminis*, *B. caribensis* and *B. phenazinium* strains and the 16 novel isolates listed in Table 1 was determined. Both quantitative and qualitative differences in cellular fatty acid composition were observed between the strains investigated (Table 3). The predominant fatty acids in all strains investigated were 16:0, 18:1 ω7*c*, summed feature 2 (comprising 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain-length value of 10·928, or 12:0 ALDE, or any combinations of these fatty acids) and summed feature 3 (comprising 16:1 ω7*c* or 15 iso 2-OH or both).

Phenotypic characterization

We determined the phenotypic characteristics of all *B. graminis*, *B. caribensis* and *B. phenazinium* strains and the 16 novel isolates listed in Table 1. The results are shown in Table 4.

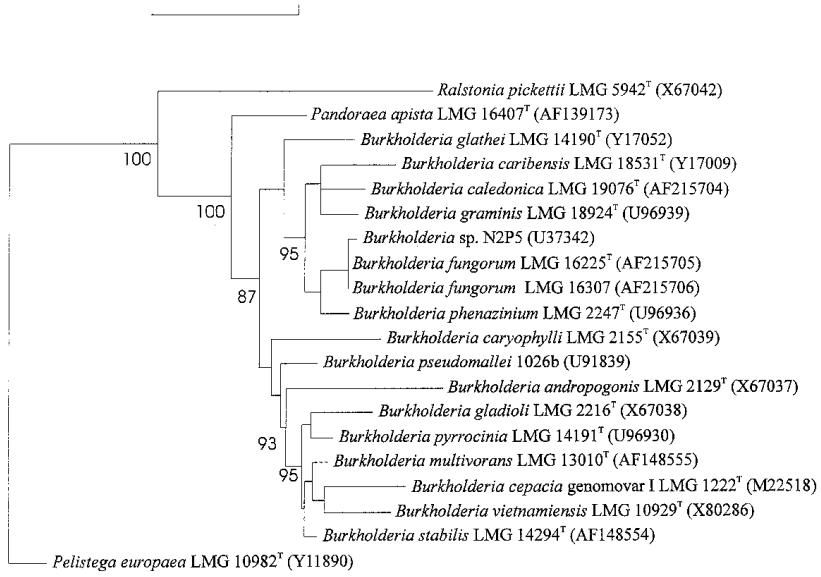

Fig. 3. Neighbour-joining tree showing the phylogenetic position of *Burkholderia fungorum* and *Burkholderia caledonica* within the genus *Burkholderia*, based on 16S rDNA sequence comparisons. *Pelistega europaea* LMG 10982^T (accession no. Y11890) was used as an outgroup in this analysis. Bar, 10 % sequence dissimilarity.

Table 2. DNA–DNA binding values and G+C content of all *Burkholderia* strains examined

Strain	Protein-electrophoretic cluster	G+C content (mol %)	DNA binding (%) with strain:										
			1	2	3	4	5	6	7	8	9	10	11
1. *B. graminis* LMG 18924^T	VII	62·6	100										
2. *B. graminis* LMG 18947	VII	63·2	99	100									
3. *B. caribensis* LMG 18531^T	V	62·8	14	16	100								
4. *B. caribensis* LMG 18532	V	62·8			95	100							
5. *B. phenazinium* LMG 2247^T	II	62·5	29	16	11	13	100						
6. *B. fungorum* LMG 16225^T	I	62·2	41		19	20	26	100					
7. *B. fungorum* LMG 18810	III	62·1	32				28	97	100				
8. *B. fungorum* R-9502	III	61·9			14			85		100			
9. *B. fungorum* R-8655	III	ND						90		87	100		
10. *B. caledonica* LMG 19076^T	VI	62·2	48	31	16		24	27	28	24	26	100	
11. *B. caledonica* LMG 19077	VI	62·2	37	34			18	30				100	100

ND, Not determined.

DISCUSSION

In the present study, we used a polyphasic taxonomic approach, including 16S rDNA sequencing, DNA–DNA hybridization, extensive biochemical characterization and cellular fatty acid and protein analysis, to determine the relationships and the phylogenetic position of 16 isolates from the environment, animals and human clinical samples.

Characterization of *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov.

It has been reported that SDS-PAGE of whole-cell proteins is a good screening method for the differentiation of *Burkholderia* species (Vandamme *et al.*, 1997). In the present study, *B. graminis*, *B. phenazinium*, *B. caribensis* and *B. glathei* reference strains each formed separate clusters, while reference strains of all other *Burkholderia* species investigated occupied distinct positions in the dendrogram obtained after numerical analysis of the whole-cell protein profiles (Fig. 1). Numerical analysis and visual comparison of the protein profiles of the 16 isolates suggested that at least two different groups were present. To determine the exact degree of genetic relatedness of strains belonging to the different protein-electrophoretic clusters, DNA–DNA hybridizations were performed. The results showed that strains belonging to protein-electrophoretic cluster VI formed a single species. Strain LMG 16225^T (cluster I) exhibited high (85–97 %) DNA–DNA binding towards strains LMG 18810, R-8655 and R-9502 (cluster III), and therefore these two clusters can be considered as two electrophoretic types of a single species. The protein patterns of strains from the two clusters were very similar, but,

Table 3. Fatty acid composition of the strains studied

Values are mean percentages of total fatty acids with standard deviations. The numbers of strains tested are shown in parentheses. Summed feature 2 comprises 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain-length value of 10·928, or 12:0 ALDE, or any combination of these fatty acids. Summed feature 3 comprises 16:1 $\omega 7c$ or 15 iso 2-OH or both. Fatty acids for which the mean amount in all taxa was less than 1 % are not given. TR, Trace amount (< 1 %).

Fatty acid	*B. fungorum* (9)	*B. caledonica* (7)	*B. graminis* (4)	*B. caribensis* (2)	*B. phenazinium* (2)
14:0	4·6 ± 0·1	4·7 ± 0·2	4·6 ± 0·0	4·2 ± 0·0	5·1 ± 0·5
16:0	14·7 ± 0·9	13·6 ± 1·6	14·4 ± 4·7	19·1 ± 0·6	15·7 ± 2·7
17:0 cyclo	5·1 ± 1·6	8·4 ± 1·5	6·9 ± 1·8	10·0 ± 0·2	8·4 ± 0·5
16:12 OH	3·5 ± 0·7	2·7 ± 0·4	5·3 ± 2·2	1·7 ± 0·1	3·0 ± 1·8
16:0 2-OH	3·6 ± 0·5	2·4 ± 0·4	3·7 ± 1·6	2·2 ± 0·3	2·0 ± 1·0
16:0 3-OH	5·6 ± 0·5	6·0 ± 0·4	7·0 ± 0·3	4·6 ± 0·4	4·2 ± 2·0
18:1 $\omega 7c$	35·6 ± 2·1	34·2 ± 1·7	26·5 ± 0·9	33·8 ± 0·4	30·1 ± 0·1
19:0 cyclo $\omega 8c$	2·5 ± 0·7	3·7 ± 0·7	3·3 ± 0·9	5·3 ± 0·1	6·3 ± 0·1
18:12-OH	1·7 ± 0·2	1·1 ± 0·3	1·8 ± 0·3	TR	1·6 ± 1·0
Summed feature 2	8·1 ± 1·1	7·4 ± 0·9	9·8 ± 1·7	6·1 ± 0·6	7·3 ± 0·7
Summed feature 3	13·6 ± 1·9	14·5 ± 1·8	15·7 ± 3·0	11·2 ± 0·2	12·6 ± 0·1

because of distortions of the protein patterns of strains of cluster III, the strains grouped into different clusters. 16S rDNA sequence analysis of representative strains of the two genomic groups (LMG 16225^T, LMG 16307 and LMG 19076^T) showed that they formed a stable phylogenetic clade together with *B. graminis*, *B. phenazinium* and *B. caribensis* (internal similarity levels were greater than 96·0 % and the bootstrap value was 95 %). Other closely related species included *B. glathei* and *B. caryophylli*, but similarities to these species were below 95·7 %. Similarity levels of members of the *B. graminis* clade towards the type species (*B. cepacia*) were between 92·4 and 93·8 %. These data indicated unambiguously that protein-electrophoretic clusters I and III and cluster VI represent two novel species within the genus *Burkholderia*, for which we propose the names *B. fungorum* sp. nov. and *B. caledonica* sp. nov.

Identification of *B. fungorum* and *B. caledonica*

Figs 1 and 2 illustrate the fact that *B. fungorum* and *B. caledonica* are characterized by different whole-cell protein profiles. However, the presence of distortions in the patterns, similar to the distortions reported for other *Burkholderia* and *Ralstonia* strains (Vandamme *et al.*, 1997, 1999; Coenye *et al.*, 1999b), hinders the identification of strains solely on the basis of numerical analysis. Therefore, visual comparison of the protein profiles is essential to avoid misidentification. Both quantitative and qualitative differences occur in the fatty acid compositions of the different species investigated (Table 3). In particular, the relatively small amount of 18:1 $\omega 7c$ in *B. graminis* allows it to be differentiated from *B. fungorum* and *B. caledonica*. The absence of significant amounts of 18:1 2-OH in *B. caribensis* separates it from *B. graminis*, *B. fungorum* and *B. caledonica*. In general, however, the differences in fatty acid composition are small, and it is questionable whether they are sufficient to enable identification of strains to the species level. Differential biochemical tests are summarized in Table 4. The most important tests for differentiating *B. fungorum*, *B. caledonica*, *B. graminis*, *B. caribensis* and *B. phenazinium* are as follows: the assimilation of trehalose, citrate, DL-norleucine, adipate and sucrose; nitrate reduction; growth in the presence of 0·5 % NaCl; and β-galactosidase activity.

Taxonomic position of *Burkholderia* sp. strains EN-B9, EN-B3, N2P6, N2P5, N3P2, Dha-54, VUN10013 and LB400

During comparison of the 16S rDNA sequences of representatives of *B. fungorum* and *B. caledonica* with available 16S rRNA sequences of other representatives of the *β-Proteobacteria*, relatively high similarities were noted towards *Burkholderia* sp. strains EN-B9 (GenBank accession no. AF074712), EN-B3 (AF074711), N2P6 (U37343), N2P5 (U37342), N3P2 (U37344), Dha-54 (AJ011508), VUN10013 (AF068011) and LB400 (U86373). All of these strains were isolated during studies on the biodegradation of xenobiotics. Strain N2P5 was isolated from a polycyclic-aromatic-hydrocarbon-contaminated soil enriched with phenanthrene (Mueller *et al.*, 1997) and shows more than 99·5 % 16S rDNA sequence similarity to *B. fungorum* strains (Fig. 3). This strongly suggests that strain N2P5 belongs to *B. fungorum*. A polyphasic taxonomic study, including DNA–DNA hybridizations, will be required to clarify the exact relationships between these strains and *B. graminis*, *B. caribensis*, *B. phenazinium*, *B. fungorum* and *B. caledonica*. The close phylogenetic relationships between these strains and *B. graminis*, *B. caribensis*, *B. phenazinium*, *B. fungorum* and *B. caledonica* suggest that other strains of the *B. graminis* clade might have similar useful xenobiotic-compound-degrading properties.

Table 4. Phenotypic characteristics of *B. fungorum* and *B. caledonica* and related species

The numbers of strains tested are shown in parentheses. Characteristics are scored as: +, all strains tested gave a positive reaction; −, none of the strains tested gave a positive reaction; v (+), result is strain dependent and the type strain is positive; v (−), result is strain dependent and the type strain is negative. The following features are present in all strains investigated: growth at 30 °C; hydrolysis of Tween 80; assimilation of D-glucose, D-mannose, D-mannitol, D-gluconate, phenylacetate, L-malate and DL-lactate; alkaline and acid phosphatase, C_8-ester lipase, leucine arylamidase and phosphoamidase activity. The following features are absent in all strains investigated: haemolysis; growth at 42 °C; production of fluorescent pigment; growth in the presence of 3·0, 4·5 or 6·0 % NaCl or acetamide; denitrification; nitrite reduction; aesculin hydrolysis; production of indole; production of acid or H_2S in triple-sugar-iron agar; assimilation of maltose; ornithine and lysine decarboxylase, tryptophanase, arginine dihydrolase, urease, gelatinase, DNase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosidase, α-mannosidase and α-fucosidase activity.

Characteristic	*B. caledonica* (7)	*B. fungorum* (9)	*B. graminis* (4)	*B. caribensis* (2)	*B. phenazinium* (2)
Catalase activity	+	v (+)	+	+	−
Oxidase activity	−	v (+)	v (−)	−	v (+)
Growth at 37 °C	v (−)	+	+	+	v (+)
Growth in OF medium with:					
D-Glucose	+	+	v (+)	+	v (−)
Maltose	v (+)	v (−)	−	−	v (−)
Adonitol	v (+)	v (−)	v (+)	v (+)	−
D-Fructose	+	v (+)	+	v (+)	+
D-Xylose	+	v (+)	v (+)	−	v (−)
Growth on cetrimide	−	v (+)	−	−	−
Nitrate reduction	−	+	+	−	−
Growth in the presence of:					
0·5 % NaCl	v (+)	+	v (+)	−	−
1·5 % NaCl	v (−)	v (+)	v (+)	−	−
10 % Lactose	v (+)	−	−	−	−
Assimilation of:					
Trehalose	+	−	+	−	−
L-Arginine	+	v (+)	+	+	+
DL-Norleucine	−	v (+)	−	+	−
L-Arabinose	+	v (+)	+	+	v (+)
N-Acetylglucosamine	v (+)	+	+	+	+
Caprate	v (−)	v (+)	+	+	−
Adipate	v (−)	v (+)	+	−	−
Citrate	−	+	+	+	−
Sucrose	v (−)	−	+	−	+
Activity of:					
β-Galactosidase	−	−	−	+	−
C_4-Esterase	v (−)	v (+)	v (+)	v (−)	+
C_{14}-Lipase	−	v (−)	−	−	−
Valine arylamidase	−	−	−	v (+)	v (−)
Cysteine arylamidase	v (−)	v (+)	v (+)	v (+)	−
β-Glucosidase	−	−	−	v (+)	−

Description of *Burkholderia fungorum* sp. nov.

Burkholderia fungorum (fun.go′rum. L. n. *fungus* mushroom; L. gen. pl. n. *fungorum* of fungi).

Cells are Gram-negative, non-sporulating, straight rods. Growth is observed at 30 °C and in the presence of 0·5 % NaCl, but not at 42 °C. Nitrite is not reduced. Nitrate is reduced. No DNase activity, β-galactosidase activity, urease activity, liquefaction of gelatin, aesculin hydrolysis or indole production. Assimilation of citrate but not of trehalose or sucrose. Additional characteristics are listed in Table 4. The following fatty acids are present in all strains: 14:0, 16:0, 17:0 cyclo, 16:1 2-OH, 16:0 2-OH, 16:0 3-OH, 18:1 ω7*c*, 19:0 cyclo ω8*c*, summed feature 2 (comprising 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain-length value of 10·928, or 12:0 ALDE, or any combinations of these fatty acids) and summed feature 3 (comprising 16:1 ω7*c* or 15 iso 2-OH or both). The G+C content is 61·9–62·2 mol %. *B. fungorum* strains have been isolated from fungi, animals and human clinical samples. Several isolates of this species have been isolated from the white-rot fungus *Phanerochaete chrysosporium*. These bacteria appeared to be very

closely associated with the fungus, and attempts to eliminate the bacteria were unsuccessful (Seigle-Murandi *et al*., 1996). It has been suggested that there is a symbiotic relationship between the bacteria and the fungus, since the bacteria were very efficient degraders of the aromatic compounds derived from degradation of lignin by white-rot fungi (Seigle-Murandi *et al*., 1996). Recently, several unidentified *B. cepacia*-like strains isolated from cystic fibrosis patients were also classified as *B. fungorum* (our unpublished observations).

The type strain, LMG 16225^T (= CCUG 31961^T), was isolated from the white-rot fungus *P. chrysosporium*. The type strain has the following additional characteristics: growth in O/F medium with fructose and xylose, on cetrimide and in the presence of 1·5% NaCl; assimilation of L-arginine, DL-norleucine, L-arabinose, caprate and adipate; C_4-esterase and cysteine arylamidase activity; G+C content of 62 mol%.

Description of *Burkholderia caledonica* sp. nov.

Burkholderia caledonica (ca.le.do′nica. L. n. *Caledonia* Latin name for the Scottish Highlands; L. adj. *caledonica* of Scotland, from where the strains were isolated).

Cells are Gram-negative, non-sporulating, straight rods. Catalase activity is present. Growth is observed at 30 °C, but not at 42 °C. Nitrate and nitrite are not reduced. No DNase activity, β-galactosidase activity, liquefaction of gelatin, aesculin hydrolysis or indole production. Assimilation of trehalose but not citrate. Additional characteristics are listed in Table 4. The following fatty acids are present in all strains: 14:0, 16:0, 17:0 cyclo, 16:1 2-OH, 16:0 2-OH, 16:0 3-OH, 18:1 ω7*c*, 19:0 cyclo ω8*c*, summed feature 2 (comprising 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain-length value of 10·928, or 12:0 ALDE, or any combinations of these fatty acids) and summed feature 3 (comprising 16:1 ω7*c* or 15 iso 2-OH or both). *B. caledonica* strains have been isolated from the rhizosphere.

The type strain, LMG 19076^T (= CCUG 42236^T), was isolated from the rhizosphere. The type strain has the following additional characteristics: growth in O/F medium with adonitol and maltose and in the presence of 10% lactose and 0·5% NaCl; assimilation of *N*-acetylglucosamine; no assimilation of caprate, adipate or sucrose; no C_4-esterase or cysteine arylamidase activity; G+C content of 62 mol%.

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