International Journal of Systematic and Evolutionary Microbiology (2015), **65**, 2265–2271 **DOI** 10.1099/ijs.0.000251

Burkholderia stagnalis sp. nov. and *Burkholderia territorii* sp. nov., two novel *Burkholderia cepacia* complex species from environmental and human sources

Birgit De Smet,[1,2] Mark Mayo,[3] Charlotte Peeters,[2] James E. A. Zlosnik,[4] Theodore Spilker,[5] Trevor J. Hird,[4] John J. LiPuma,[5] Timothy J. Kidd,[6] Mirjam Kaestli,[3] Jennifer L. Ginther,[7] David M. Wagner,[7] Paul Keim,[7] Scott C. Bell,[6,8,9] Jan A. Jacobs,[1,10] Bart J. Currie[3] and Peter Vandamme[2]

Correspondence
Peter Vandamme
Peter.Vandamme@UGent.be

[1]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

[2]Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium

[3]Global and Tropical Health Division, Menzies School of Health Research, Darwin, Northern Territory, Australia

[4]Centre for Understanding and Preventing Infection in Children, Department of Paediatrics, University of British Columbia, Vancouver, BC, Canada

[5]Department of Paediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA

[6]Queensland Children's Medical Research Institute, University of Queensland, Brisbane, Queensland, Australia

[7]Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA

[8]Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Queensland, Australia

[9]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

[10]Department of Microbiology and Immunology, University of Leuven, Belgium

Nine *Burkholderia cepacia* complex (Bcc) bacteria were isolated during environmental surveys for the ecological niche of *Burkholderia pseudomallei*, the aetiological agent of melioidosis, in the Northern Territory of Australia. They represented two multi-locus sequence analysis-based clusters, referred to as Bcc B and Bcc L. Three additional environmental and clinical Bcc B isolates were identified upon deposition of the sequences in the PubMLST database. Analysis of the concatenated nucleotide sequence divergence levels within both groups (1.4 and 1.9 %, respectively) and towards established Bcc species (4.0 and 3.9 %, respectively) demonstrated that the two taxa represented novel Bcc species. All 12 isolates were further characterized using 16S rRNA and *recA* gene sequence analysis, RAPD analysis, DNA base content determination, fatty acid methyl ester analysis and biochemical profiling. Analysis of *recA* gene sequences revealed a remarkable diversity within each of these taxa, but, together, the results supported the affiliation of the two taxa to the Bcc. Bcc B strains can be differentiated from most other Bcc members by the assimilation of maltose. Bcc L strains can be differentiated from other Bcc members by the absence of assimilation of *N*-acetylglucosamine. The names *Burkholderia stagnalis* sp. nov. with type strain LMG 28156^{T} (=CCUG 65686^{T}) and *Burkholderia territorii* sp. nov. with type strain LMG 28158^{T} (=CCUG 65687^{T}) are proposed for Bcc B and Bcc L bacteria, respectively.

Abbreviations: Bcc, *Burkholderia cepacia* complex; CF, cystic fibrosis; MLSA, multi-locus sequence analysis; RAPD, random amplified polymorphic DNA; ST, sequence type.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 28156^{T} and LMG 28158^{T} are LK023502 and LK023503, respectively. Those for the *recA* gene sequences of strains R-52235, LMG 28156^{T}, R-52237, R-52238, LMG 28157, R-52095, R-52096, R-52240, LMG 28158^{T}, R-52242, LMG 28159 and R-52244 are LK023504–LK023515, respectively.

Eight supplementary figures are available with the online Supplementary Material.

000251 © 2015 IUMS *Printed in Great Britain*

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

The *Burkholderia cepacia* complex (Bcc) is a versatile group of closely related bacteria that are ubiquitous in nature (Coenye & Vandamme, 2003; Peeters *et al.*, 2013; Vandamme & Peeters, 2014; Vanlaere *et al.*, 2009). Some Bcc strains show biotechnological potential for bio-control, bioremediation and plant growth promotion, whereas other strains are pathogens for plants and immunocompromised individuals such as those with cystic fibrosis (CF). Bcc strains are also common nosocomial pathogens (Vonberg & Gastmeier, 2007). The Bcc comprises, at the time of writing, 18 validly named species (Peeters *et al.*, 2013), which exhibit a high degree of 16S rRNA (98–100 %) and *recA* (94–95 %) gene sequence similarity, moderate levels of DNA–DNA hybridization (30–50 %) (Coenye *et al.*, 2001) and whole-genome average nucleotide identity values of between 85.04 and 89.92 % (Vanlaere *et al.*, 2009). Protein encoding genes applied in a multilocus sequence analysis (MLSA) approach have a high discriminatory power for the identification of Bcc isolates, and 3 % concatenated sequence divergence has been validated as a threshold level for species differentiation (Peeters *et al.*, 2013; Vanlaere *et al.*, 2009). The application of this threshold level to analyse publicly available MLST data revealed the presence of at least another 16 novel Bcc species that await formal description (Vandamme & Peeters, 2014).

Nine Bcc bacteria were isolated in ongoing environmental soil and water sampling surveys for the ecological niches of *Burkholderia pseudomallei*, the aetiological agent of melioidosis, in the tropical Northern Territory of Australia. As part of the Darwin prospective melioidosis study (Currie *et al.*, 2010) household yards, gardens and domestic water supplies and other locations in Darwin city and its rural surrounds have been opportunistically sampled over the last two decades, with recovered *B. pseudomallei* and other species of the genus *Burkholderia* characterized and stored. Environmental sampling and bacterial isolation methods have been described previously (Kaestli *et al.*, 2012; Mayo *et al.*, 2011).

These nine isolates represented two unique MLSA clusters within the Bcc, referred to earlier as clusters Bcc B and Bcc L (Vandamme & Peeters, 2014). Upon deposition of the sequences in the Bcc PubMLST database (http://pubmlst.org/bcc/), three additional Bcc B isolates were identified: one environmental soil isolate from North Carolina, USA, one clinical isolate from a respiratory specimen (tracheal aspirate) of a native American non-CF adult patient from North Carolina (we were unable to verify if infection could have been acquired abroad) and one isolate from a sputum sample of a patient with CF from Australia (Table 1).

All isolates were preserved in MicroBank vials at −80 °C. They were grown aerobically on tryptone soya agar (Oxoid) and incubated at 30 °C for further characterization, except when mentioned otherwise. For PCR experiments, DNA was prepared by alkaline lysis as described by Storms *et al.* (2004).

MLSA was performed using standard protocols (Peeters *et al.*, 2013; Spilker *et al.*, 2009). Nucleotide sequences of

Table 1. Studied isolates, showing their source, ST and allelic profile

Strain*	Other strain designations†	Source‡ (country, year of isolation)	ST	Allelic profile Bcc MLST						
				atpD	*gltB*	*gyrB*	*recA*	*lepA*	*phaC*	*trpB*
B. stagnalis sp. nov.										
R-52235	MSMB049; FC1736	Soil (Australia, 2006)	787	170	197	564	345	300	213	381
LMG 28156^T	CCUG 65686^T; MSMB050^T; FC1737^T	Soil (Australia, 2006)	787	170	197	564	345	300	213	381
R-52237	MSMB085; FC1738	Soil (Australia, 2007)	789	328	379	399	347	300	213	196
R-52238	MSMB086; FC1739	Soil (Australia, 2007)	789	328	379	399	347	300	213	196
LMG 28157	MSMB087; FC1740	Soil (Australia, 2007)	789	328	379	399	347	300	213	196
R-52095	HI3541; FC1742	Soil (USA, 2005)	865	205	406	420	366	245	191	247
R-52096	AU7314; FC1741	Tracheal aspirate, non-CF (USA, 2004)	519	170	197	399	190	300	213	196
R-52240	MSMB2195; QLD037; FC1812	CF sputum (Australia, 2008)	690	300	345	511	319	300	274	351
B. territorii sp. nov.										
LMG 28158^T	CCUG 65687^T; MSMB110^T; FC1743^T	Water (Australia, 2003)	791	233	381	567	348	395	304	384
R-52242	MSMB117; FC1744	Water (Australia, 2003)	792	329	382	568	349	396	305	385
LMG 28159	MSMB138; FC1745	Water (Australia, 2003)	794	331	384	570	351	398	306	386
R-52244	MSMB139; FC1746	Water (Australia, 2003)	794	331	384	570	351	398	306	386

*LMG, BCCM/LMG Bacteria Collection, Laboratory of Microbiology, Ghent University, Ghent, Belgium.
†CCUG, Culture Collection, University of Göteborg, Sweden; MSMB, Menzies School of Health Research, Darwin, Australia.
‡CF, Cystic fibrosis patient.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

each allele, allelic profiles and sequence types (STs) for all isolates represented five STs for cluster Bcc B isolates and three STs for cluster Bcc L isolates, and are available on the Bcc PubMLST website (http://pubmlst.org/bcc) (Jolley & Maiden, 2010). The mean number of nucleotide substitutions per site (i.e. the percentage of divergence of concatenated allele sequences) between established (June 2014) and the newly proposed Bcc species was calculated using the program DnaSP v5.10 (Librado & Rozas, 2009) based on the Jukes–Cantor method (Jukes & Cantor, 1969). A phylogenetic tree of the concatenated sequences (2773 bp) of seven housekeeping gene fragments, namely *atpD* (443 bp), *gltB* (400 bp), *gyrB* (454 bp), *recA* (393 bp), *lepA* (397 bp), *phaC* (385 bp) and *trpB* (301 bp), was constructed using MEGA5 (Tamura *et al.*, 2011).

Within cluster Bcc B ($n=5$) the concatenated allele sequence divergence was 1.4 ± 0.6 %, and the average divergence towards its nearest neighbour, *Burkholderia ubonensis*, was 4.0 ± 0.8 %. Within cluster Bcc L ($n=3$) the concatenated allele sequence divergence was 1.9 ± 0.7 %, and the average divergence towards its nearest neighbour, *Burkholderia diffusa*, was 3.9 ± 0.9 % (data not shown). These concatenated sequence divergence values demonstrate that the two clusters represent novel species within the Bcc (Peeters *et al.*, 2013; Vanlaere *et al.*, 2009).

When a non-Bcc outgroup species (*Burkholderia fungorum*) was included in the phylogenetic analysis of the concatenated sequences, cluster Bcc B and cluster Bcc L isolates constituted two clusters supported by high bootstrap values of 93 and 99 %, respectively (Fig. 1; see also Vandamme & Peeters, 2014). Yet a bifurcation was apparent in each cluster and the removal of the outgroup taxon reinforced the differences between these subgroups in the resulting phylogenetic trees: Figs S1–S7 (available in the online Supplementary Material) show the results of phylogenetic analyses based on single gene sequences. Among Bcc B isolates, R-52095 had *gltB*, *lepA* and *trpB* gene sequences that were very similar to those of the remaining cluster Bcc B strains, whereas its *gyrB*, *phaC* and, to an even greater extent, *atpD* and *recA* gene sequences were divergent from those of the remaining cluster Bcc B strains. Among the cluster Bcc L strains, isolates LMG 28159 and R-52244 differed from LMG 28158^{T} and R-52242 in particular for the *recA*, *trpB* and, to a lesser extent, *lepA* gene sequences, whereas the *atpD*, *gltB*, *gyrB* and *phaC* gene sequences were highly similar (Figs S1–7).

Random amplified polymorphic DNA (RAPD) analysis of all isolates was performed with primer 270 as described by Mahenthiralingam *et al.* (1996) and profiles were compared visually (data not shown). For the eight Bcc B isolates, five different RAPD profiles where observed, corresponding to the five STs. For the four Bcc L isolates, four different RAPD profiles were observed; isolates LMG 28159 and R-52244, which represented a single ST, had only slightly different RAPD profiles (data not shown).

The nearly complete sequences of the 16S rRNA gene of strains LMG 28156^{T} (1485 bp) and LMG 28158^{T} (1443 bp) were obtained as described previously (Peeters *et al.*, 2013). Pairwise comparison of these sequences, as determined with the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon, (Kim *et al.*, 2012), with those of type strains of other Bcc species revealed similarity levels of between 98.43 and 99.66 % for the former and between 98.89 and 100 % for the latter (data not shown). Similarity levels towards non-Bcc *Burkholderia* species were in the range 94.28–98.98 %. For both strains the highest similarity values were obtained towards the type strain of *Burkholderia glumae*.

Sequences of the *recA* gene were amplified using forward primer 5′-AGGACGATTCATGGAAGAWAGC-3′ and reverse primer 5′-GACGCACYGAYGMRTAGAACTT-3′ (Spilker *et al.*, 2009). Sequence assembly was performed using BioNumerics v5.10 (Applied Maths). Sequences (540–660 bp) were aligned based on amino acid sequences using Muscle (Edgar, 2004) in MEGA5 (Tamura *et al.*, 2011). Phylogenetic analysis was conducted in MEGA5 (Tamura *et al.*, 2011). All positions containing gaps and missing data were eliminated, resulting in a total of 473 positions in the final dataset. All Bcc B isolates except isolate R-52095, which occupied a distinct position in the *recA* gene tree, formed a coherent *recA* gene cluster with *Burkholderia pseudomultivorans* as its nearest neighbour (Fig. S8). These results confirmed and extended the considerable differences observed in the 393 bp *recA* gene fragment of the MLSA scheme (Fig. S4). Similarly, the bifurcation of the four Bcc L strains was confirmed in the 473 bp based analysis (Fig. S8).

For determination of the DNA base composition, high-molecular-mass DNA was prepared as described by Pitcher *et al.* (1989). DNA was enzymically degraded into nucleosides as described by Mesbah & Whitman (1989). The nucleoside mixture obtained was separated using a Waters Breeze HPLC system and XBridge Shield RP18 column thermo-stabilized at 37 °C. The solvent was 0.02 M $NH_4H_2PO_4$ (pH 4.0) with 1.5 % (v/v) acetonitrile. Non-methylated lambda phage (Sigma) and *Escherichia coli* LMG 2093 DNA were used as calibration reference and control, respectively. The DNA G+C content of strains LMG 28156^{T} and LMG 28158 ^{T} was both 67 mol%, which corresponded with that of other Bcc species (66–69 mol%) (Vandamme & Dawyndt, 2011).

For fatty acid methyl ester analysis, all strains were grown on tryptone soya agar (BD) at 28 °C for 24 h. A loopfull 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). The following fatty acid components were detected in Bcc B and Bcc L isolates, respectively: $C_{14:0}$ (4.08 ± 1.3 and 4.3 ± 0.3 %),

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

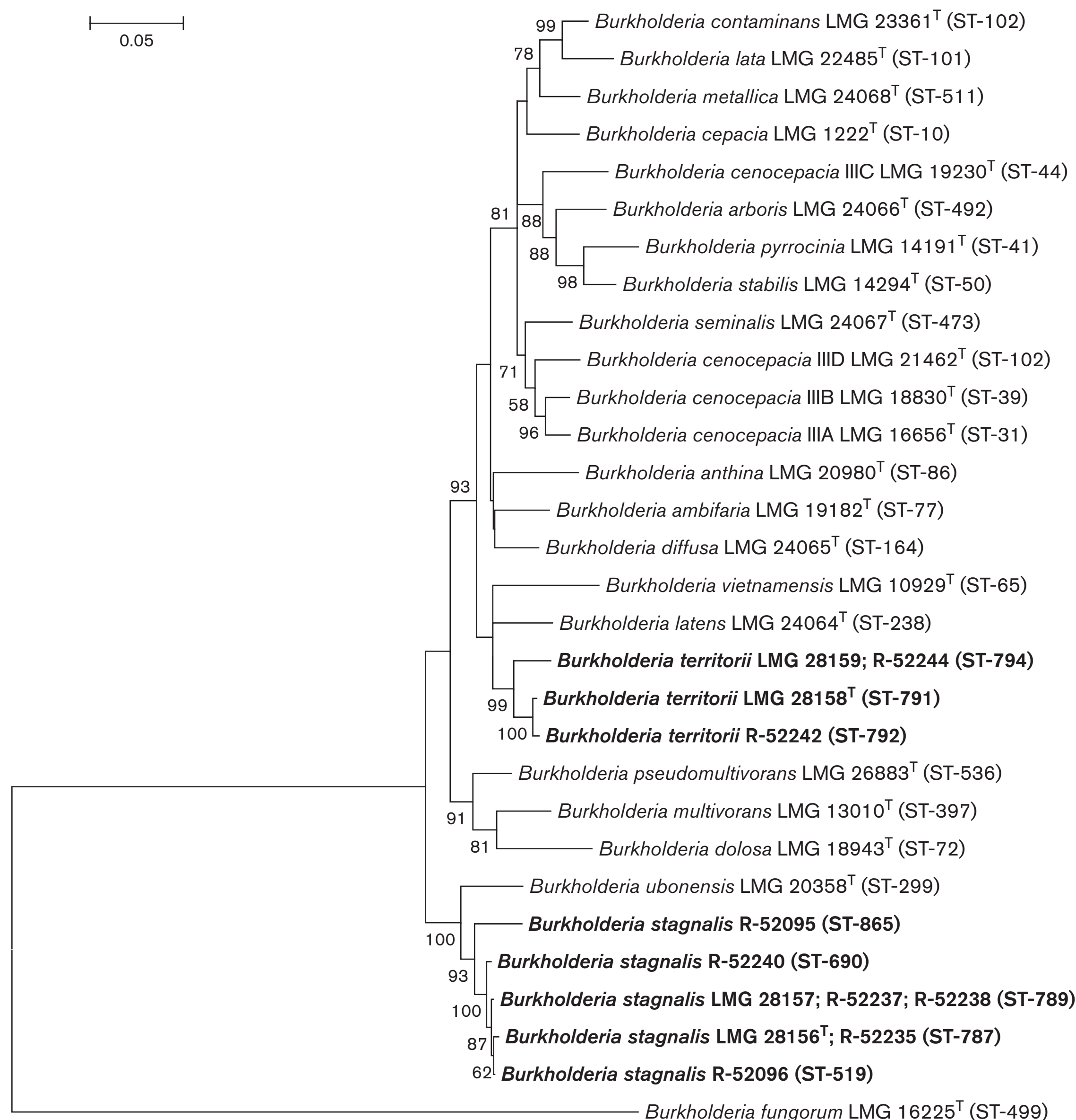

Fig. 1. Phylogenetic tree based on the concatenated sequences (2773 bp) of seven housekeeping gene fragments [*atpD* (443 bp), *gltB* (400 bp), *gyrB* (454 bp), *recA* (393 bp), *lepA* (397 bp), *phaC* (385 bp) and *trpB* (301 bp)] of established Bcc species and *Burkholderia stagnalis* sp. nov. and *Burkholderia territorii* sp. nov. strains. The bootstrap consensus tree, inferred from 1000 replicates, was reconstructed using the maximum-likelihood method based on the general time reversible model. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches if greater than 50 %. A discrete gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter=0.3623)] and allowed for some sites to be evolutionarily invariable [(+I), 51.7996 % sites]. The analysis involved 30 nt sequences. Evolutionary analyses were conducted in MEGA5.The sequence of *B. fungorum* LMG 16225^T was used as an outgroup. Bar, 0.05 substitutions per site.

$C_{16:0}$ (18.7 ± 0.9 and 19.0 ± 0.9 %), $C_{16:0}$ 2-OH (1.5 ± 0.5 and 1.2 ± 0.6 %), $C_{16:0}$ 3-OH (8.4 ± 1.0 and 7.1 ± 0.9 %), $C_{16:1}$ 2-OH (1.9 ± 0.6 and 1.0 ± 0.4 ;), $C_{17:0}$ cyclo (10.2 ± 4.2 and 6.1 ± 3.2 %), $C_{18:1}\omega7c$ (24.0 ± 4.5 and 30.1 ± 1.7 %), $C_{19:0}$ cyclo $\omega8c$ (4.4 ± 2.4 and 2.1 ± 1.3 %), $C_{18:0}$ 2-OH (2.8 ± 0.7 and 2.5 ± 1.0 %), summed feature 2 (comprising $C_{14:0}$ 3-OH, iso-$C_{16:1}$ I, an unidentified fatty acid with equivalent chain-length of 10.928 or $C_{12:0}$ ALDE or any combination of these) (10.3 ± 1.7 and 10.8 ± 3.3 %) and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) (12.2 ± 4.9 and 15.2 ± 4.0 %). These cellular fatty acid profiles are highly similar to those of other Bcc species and *Burkholderia gladioli* (Stead, 1992).

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

Biochemical characterization of all isolates was performed as described by Henry *et al.* (2001) and a summary of the results can be found in Table 2 and in the species descriptions below. Biochemically, all Bcc B and Bcc L strains are similar to other Bcc bacteria. Bcc B strains can be differentiated from other Bcc species (except for *B. ubonensis*) by the assimilation of maltose. The only difference noted between isolate R-52095 and the remaining Bcc B isolates is the presence of β-haemolysis. Bcc L strains can be differentiated from other Bcc species by the absence of assimilation of *N*-acetylglucosamine. The two Bcc L *recA* gene clusters differed in nitrate reduction capacity.

In conclusion, MLSA demonstrated that 12 Bcc isolates represented two novel species within the Bcc. DNA base content determination, 16S rRNA gene sequence analysis and fatty acid methyl ester analysis confirmed that these isolates conformed to the general characteristics of the genus *Burkholderia*. The two novel species consisted of two *recA* gene lineages that were phenotypically different; yet assimilation of maltose and of *N*-acetylglucosamine could be used to distinguish these species from other Bcc species. We therefore propose to formally classify Bcc B and Bcc L strains as representing the novel species *Burkholderia stagnalis* sp. nov. with strain LMG 28156^{T} (=CCUG 65686^{T}) as the type strain, and *Burkholderia territorii* sp. nov. with strain LMG 28158^{T} (=CCUG 65687^{T}) as the type strain, respectively.

Description of *Burkholderia stagnalis* sp. nov.

Burkholderia stagnalis (stag.na'lis. L. n. *stagnum* pond, lagoon, lake; -*alis* adjective forming suffix; N.L. fem. adj. *stagnalis* pertaining to a lagoon).

Cells are Gram-stain-negative , aerobic, motile, non-spore-forming rods, about 0.1–0.5 μm wide and 0.6–1.7 μm long. All isolates grow at 37 °C on BSA, *Burkholderia cepacia* selective agar and MacConkey agar and do not produce any pigment. The majority (six of eight) of the isolates show medium-sized grey entire colonies after 3 days of

Table 2. Phenotypic characteristics useful for differentiation of *B. stagnalis* sp. nov. and *B. territorii* sp. nov. from members of the Bcc

Species: 1, *B. stagnalis* sp. nov.; 2, *B. territorii* sp. nov.; 3, *B. pseudomultivorans*; 4, *B. contaminans*; 5, *B. lata*; 6, *B. latens*; 7, *B. diffusa*; 8, *B. arboris*; 9, *B. seminalis*; 10, *B. metallica*; 11, *B. cepacia*; 12, *B. multivorans*; 13, *B. cenocepacia*; 14, *B. stabilis*; 15, *B. vietnamiensis*; 16, *B. dolosa*; 17, *B. ambifaria*; 18, *B. anthina*; 19, *B. pyrrocinia*; 20, *B. ubonensis*. +, >90 % of all isolates positive; v, 10–90 % positive; −, <10 % of strains positive; w, weak reaction; β, β-haemolysis; y, yellow. For *B. stagnalis* (n=8) and *B. territorii* (n=4), the number of positive reactions is indicated for strain-dependent reactions and the reaction of the type strain is given in parentheses. For characteristics of established Bcc species data were taken from the present study and Peeters *et al.* (2013).

Phenotypic characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Growth on MacConkey agar	+	+	+	+	+	+	+	+	+	+	v	+	v	+	v	+	+	+	+	+
Growth at 42 °C	5 (+)	+	+	v	−	+	v	v	+	+	v	+	v	−	+	+	v	v	v	v
Pigment	−	−	−	v	v	−	−	v	v	+ (y)	v	−	v	−	−	−	v	−	v	−
Haemolysis (sheep blood)	1 β (−)	−	−	v	−	−	−	v	−	−	−	−	−	−	v	−	v	−	v	−
Assimilation of:																				
L-Arabinose	5 (+)	+	+	+	v	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+
N-Acetylglucosamine	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+
Maltose	+	3 (−)	v	−	v	−	−	v	−	−	−	−	−	−	v	−	−	v	v	+
Adipate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	+
Phenylacetate	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidification of:																				
Maltose	+	+	+	v	+	+	+	+	+	+	v	+	v	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	w	+	+	+	+	+	v	v	+	+	+	+	+
Sucrose	7 (+)	3 (+)	v	+	v	+	+	+	+	+	+	−	+	−	+	−	+	v	+	+
Adonitol	−	+	v	+	v	+	v	+	+	+	v	+	v	+	−	+	+	v	+	−
Nitrate reduction	−	2 (−)	v	v	v	−	+	v	−	−	−	+	v	−	v	+	v	v	v	v
Activity of:																				
Lysine decarboxylase	+	+	+	+	+	+	+	v	v	+	+	v	+	+	+	−	+	v	+	−
Ornithine decarboxylase	−	−	−	−	v	−	−	+	v	−	v	−	v	+	−	−	−	−	+	−
Aesculin hydrolysis	−	−	v	v	v	−	−	−	v	+	v	−	v	−	−	–	v	−	−	−
Arginine dihydrolase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Gelatinase	7 (+)	+	−	+	v	v	v	+	+	+	v	−	v	v	−	−	+	−	+	+
β-Galactosidase	1 (−)	+	+	+	v	+	+	+	+	+	+	+	+	−	+	+	+	v	+	−

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

incubation on BSA, and the remaining two isolates show distinctive colony centres. Only one isolate (R-52095) shows β-haemolysis on blood agar. The majority of isolates (five of eight) are able to grow at 42 °C (R-52235, LMG 28156^T, R-52237, R-52238, R-52239). All isolates show slow oxidase-positive reactions and lysine decarboxylase activity, but no nitrate reduction, ornithine decarboxylase or aesculin hydrolysis activity. Furthermore, glucose, maltose, lactose and xylose are acidified, but not adonitol. Assimilation of maltose is observed for all strains. Strain-dependent reactions are observed for acidification of sucrose (negative for R-52240), gelatinase (negative for R-52096) and β-galactosidase (weakly positive for R-52096) activity. API 20NE profiles are 0057777 (six of eight isolates), 0056577 (R-52240) and 0067777 (R-52096). The following fatty acids are present in all isolates: $C_{14:0}$, $C_{16:1}$ 2-OH, $C_{16:0}$ 2-OH, $C_{18:0}$, cyclo $C_{19:0}\omega8c$ and $C_{18:1}$ 2-OH all at $<5\%$ (mean value of all isolates); and $C_{16:0}$, cyclo $C_{17:0}$, $C_{16:0}$ 3-OH, $C_{18:1}\omega7c$ and summed features 2 and 3 all at between 5 and 25% (mean value of all isolates). Isolates have been obtained from soil and human respiratory samples in Australia and the USA.

The type strain is LMG 28156^T (=CCUG 65686^T, originally collected as MSMB050^T). Phenotypic characteristics of the type strain are the same as those described above for all strains of the species. In addition, the type strain grows at 42 °C, does not show β-haemolysis on sheep blood agar, acidifies sucrose, assimilates arabinose and shows gelatinase activity. The DNA G+C content of the type strain is 67 mol%.

Description of *Burkholderia territorii* sp. nov.

Burkholderia territorii [ter.ri.to′ri.i. L. gen. n. *territorii* of the territory; referring to the (Northern) Territory of Australia, where this organism has been recovered from environmental groundwater samples].

Cells are Gram-stain-negative, aerobic, motile, non-spore-forming rods, about 0.2–0.5 µm wide and 0.5–1.7 µm long. All isolates grow at 37 °C on BSA, *Burkholderia cepacia* selective agar and MacConkey agar. They show medium-sized grey entire colonies on BSA without haemolysis, no pigment production is observed and all strains grow at 42 °C. Two isolates (R-52243 and R-52244) display mixed colonies types with small and large colonies. However, RAPD on both colony types shows identical patterns. All isolates show slow oxidase-positive reactions, lysine decarboxylase, β-galactosidase and gelatinase activity but no ornithine decarboxylase or aesculin hydrolysis activity. Acidification of glucose, maltose, lactose, xylose and adonitol is positive, with strain-dependent reaction for sucrose acidification (negative for R-52242), as well as nitrate reduction (negative for LMG 28158^T and R-52242). No assimilation of *N*-acetylglucosamine. API 20NE profiles are 0077567 (LMG 28158^T), 0077577 (R-52242) and 1077577 (R-52243 and R-52244). The following fatty acids are present in all isolates: $C_{14:0}$, $C_{16:1}$ 2-OH, $C_{16:0}$ 2-OH, $C_{18:0}$, cyclo $C_{19:0}\omega8c$ and $C_{18:1}$ 2-OH all at $<5\%$; and $C_{16:0}$, cyclo $C_{17:0}$, $C_{16:0}$ 3-OH, $C_{18:1}\omega7c$, and summed features 2 and 3 all at between 5 and 30%. Strains have been isolated from environmental groundwater samples.

The type strain is LMG 28158^T (=CCUG 65687^T, originally collected as MSMB110^T). Phenotypic characteristics of the type strain are the same as those described above for all strains of the species. In addition, the type strain reduces nitrate and acidifies sucrose. The DNA G+C content of the type strain is 67 mol%.

Acknowledgements

We thank all strain depositors who contributed to this study, Cindy Snauwaert (Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium), Evie De Brandt (Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium), Rebecca Hickman (University of British Columbia) and Kay Ramsay (Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Queensland, Australia) for technical assistance, and Glenda Harrington (Menzies School of Health Research, Darwin, Northern Territory, Australia) for assistance with environmental sampling. Collection of the environmental samples was supported by grants from the Australian National Health and Medical Research Council (605820 and 1046812). J. E. A. Z. and T. J. H. are very grateful for the support of David P. Speert and financial support provided to D. P. S. by Cystic Fibrosis Canada through an operating grant (20R32976) and support for the Canadian Burkholderia cepacia complex research and referral repository (20R40868). Initial MLSA was supported by grant funding from the Children's Health Foundation Queensland (50005). This publication made use of the *Burkholderia cepacia* complex Multi Locus Sequence Typing website (http://pubmlst.org/bcc/) developed by Keith Jolley and sited at the University of Oxford (Jolley & Maiden, 2010). The development of this site has been funded by the Wellcome Trust.

References

Coenye, T. & Vandamme, P. (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* **5**, 719–729.

Coenye, T., Vandamme, P., Govan, J. R. & LiPuma, J. J. (2001). Taxonomy and identification of the *Burkholderia cepacia* complex. *J Clin Microbiol* **39**, 3427–3436.

Currie, B. J., Ward, L. & Cheng, A. C. (2010). The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. *PLoS Negl Trop Dis* **4**, e900.

Edgar, R. C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**, 113.

Henry, D. A., Mahenthiralingam, E., Vandamme, P., Coenye, T. & Speert, D. P. (2001). Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. *J Clin Microbiol* **39**, 1073–1078.

Jolley, K. A. & Maiden, M. C. (2010). BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **11**, 595.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, **vol. 3**, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kaestli, M., Schmid, M., Mayo, M., Rothballer, M., Harrington, G., Richardson, L., Hill, A., Hill, J., Tuanyok, A. & other authors (2012). Out of the ground: aerial and exotic habitats of the melioidosis bacterium *Burkholderia pseudomallei* in grasses in Australia. *Environ Microbiol* **14**, 2058–2070.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Mahenthiralingam, E., Campbell, M. E., Henry, D. A. & Speert, D. P. (1996). Epidemiology of *Burkholderia cepacia* infection in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. *J Clin Microbiol* **34**, 2914–2920, .

Mayo, M., Kaesti, M., Harrington, G., Cheng, A. C., Ward, L., Karp, D., Jolly, P., Godoy, D., Spratt, B. G. & Currie, B. J. (2011). *Burkholderia pseudomallei* in unchlorinated domestic bore water, Tropical Northern Australia. *Emerg Infect Dis* **17**, 1283–1285.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

Peeters, C., Zlosnik, J. E., Spilker, T., Hird, T. J., LiPuma, J. J. & Vandamme, P. (2013). *Burkholderia pseudomultivorans* sp. nov., a novel *Burkholderia cepacia* complex species from human respiratory samples and the rhizosphere. *Syst Appl Microbiol* **36**, 483–489.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Spilker, T., Baldwin, A., Bumford, A., Dowson, C. G., Mahenthiralingam, E. & LiPuma, J. J. (2009). Expanded multilocus sequence typing for *Burkholderia* species. *J Clin Microbiol* **47**, 2607–2610.

Stead, D. E. (1992). Grouping of planth-pathogenic and some other Pseudomonas spp. by using cellular fatty-acid profiles. *Int J Syst Bacteriol* **42**, 281–295.

Storms, V., Van Den Vreken, N., Coenye, T., Mahenthiralingam, E., LiPuma, J. J., Gillis, M., Vandamme, P. & Vandamme, P. (2004). Polyphasic characterisation of *Burkholderia cepacia*-like isolates leading to the emended description of *Burkholderia pyrrocinia*. *Syst Appl Microbiol* **27**, 517–526.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Vandamme, P. & Dawyndt, P. (2011). Classification and identification of the *Burkholderia cepacia* complex: past, present and future. *Syst Appl Microbiol* **34**, 87–95.

Vandamme, P. & Peeters, C. (2014). Time to revisit polyphasic taxonomy. *Antonie van Leeuwenhoek* **106**, 57–65.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., van den Borre, C., Higgins, R. & other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.

Vanlaere, E., Baldwin, A., Gevers, D., Henry, D., De Brandt, E., LiPuma, J. J., Mahenthiralingam, E., Speert, D. P., Dowson, C., Vandamme, P. & Taxon, K. (2009). Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. *Int J Syst Evol Microbiol* **59**, 102–111.

Vonberg, R. P. & Gastmeier, P. (2007). Hospital-acquired infections related to contaminated substances. *J Hosp Infect* **65**, 15–23.

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Sat, 15 Dec 2018 16:57:54