***Burkholderia caribensis* sp. nov., an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique**

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Twenty-one exopolysaccharide-producing strains were isolated from the 5–20 µm fraction of a vertisol in the south-east of the island of Martinique in the French West Indies. Although these strains were phenotypically identified as *Burkholderia cepacia* or as *Burkholderia glathei* using BIOLOG microplates, they did not cluster genotypically by amplified rDNA restriction analysis (ARDRA) with any described *Burkholderia* species. A phylogenetic analysis revealed that the *rrs* (16S rDNA) sequences of three representative strains clustered in a single branch within the genus *Burkholderia* and distantly from all of the previously described species of *Burkholderia* for which *rrs* sequences were available. DNA–DNA hybridization data as well as phenotypic analyses indicated that the 21 isolates represented a single and new species for which the name *Burkholderia caribensis* sp. nov. is proposed (type strain MWAP64^T = LMG 18531^T).

Keywords: *Burkholderia caribensis* sp. nov., vertisol microaggregate, *rrs* analysis, DNA–DNA hybridization, phenotypic analysis

INTRODUCTION

Almost 80 years after the first description of the genus *Pseudomonas* (Migula, 1894), this genus was divided into five groups (Palleroni *et al.*, 1973). This division was based mainly on extensive phenotypic studies, and DNA–rRNA and DNA–DNA hybridization experiments (De Vos *et al.*, 1985; Palleroni *et al.*, 1973; Palleroni, 1984, 1992). In a recent review on analysis of *rrs* (16S rDNA) sequences, it was shown that several sequences of species formerly described as *Pseudomonas* must be distributed to three of the five subclasses of the *Proteobacteria* (Kerstens *et al.*, 1996). The γ -subclass includes the authentic genus *Pseudomonas* that contains the type species *Pseudomonas aeruginosa* and related species such as *Pseudomonas fluorescens*.

The α -subclass includes several misnamed *Pseudomonas* species. Two of these species were transferred to the new genus *Brevundimonas* (Segers *et al.*, 1994) as *Brevundimonas diminuta* and *Brevundimonas vesicularis*, and *Pseudomonas paucimobilis* was transferred to the genus *Sphingomonas* (Yabuuchi *et al.*, 1990). The family *Comamonadaceae*, including the genera *Acidovorax*, *Comamonas* and *Hydrogenophaga*, belongs to the β -subclass together with the genera *Burkholderia* (Yabuuchi *et al.*, 1992) and *Ralstonia* (Yabuuchi *et al.*, 1995).

Using a polyphasic taxonomy approach (Brett *et al.*, 1998; Gillis *et al.*, 1995; Urakami *et al.*, 1994; Vandamme *et al.*, 1997; Viallard *et al.*, 1998; Yabuuchi *et al.*, 1992; Zhao *et al.*, 1995), the genus *Burkholderia* was recently restricted to 17 species, including a newly described root-associated nitrogen-fixing species *Burkholderia vietnamiensis* (Gillis *et al.*, 1995), other root-associated species *Burkholderia vandii* (Urakami *et al.*, 1994) and *Burkholderia graminis* (Viallard *et al.*, 1998), and the recently described *Burkholderia multivorans* (Vandamme *et al.*, 1997) and *Burkholderia thailan-*

Abbreviations: ARDRA, amplified rDNA restriction analysis; EPS, exopolysaccharide.

GenBank accession numbers for the *rrs* sequences reported in this paper are Y17009 (strain MWAP64^T), Y17010 (strain MWAP71), Y17011 (strain MWAP84) and Y17052 (*B. glathei* strain LMG 14190^T).

densis (Brett *et al.*, 1998). This genus is clearly separated from *Ralstonia*, which includes *Ralstonia pickettii* (type species), *Ralstonia* (formerly *Pseudomonas*) *solanacearum* and *Ralstonia* (formerly *Alcaligenes*) *eutropha* (Yabuuchi *et al.*, 1995).

During the last 10 years, several laboratories have focused their attention on *Burkholderia cepacia* (type species) due to its abundance in soil, in the plant rhizosphere and in clinical habitats (Hebbar *et al.*, 1992a; Yohalem & Lorbeer, 1994). These studies were stimulated by a semi-selective medium (PCAT) (Lumsden & Sasser, 1986) which facilitates the screening of strains for their ability to degrade polycyclic aromatic hydrocarbons (Mueller *et al.*, 1997) or for their potency to antagonize soil-borne phytopathogens (Hebbar *et al.*, 1992b). Besides the ability to grow on PCAT medium, phenotypic identification (e.g. by GC-FAME and BIOLOG microplates) and genotypic identification (e.g. by *rrs* sequencing and/or DNA-DNA hybridization) are needed to clarify the taxonomic position of many strains grouped with *B. cepacia* (Mueller *et al.*, 1997).

The objective of this study was to identify bacterial populations that are responsible for the formation of microaggregates in a vertisol on the island of Martinique. Vertisols are characterized by a very high clay content. In these soils, microaggregation results mainly from the fact that bacterial colonies embedded in their exopolysaccharides (EPS) are covered with clay particles (Tisdall, 1994). To isolate these microaggregate-forming bacteria, we prepared a soil fraction rich in microaggregates (5–20 µm) and selected bacteria for their EPS production. The phylogenetic position of the major EPS-producing bacterial species isolated from this soil fraction was determined according to *rrs* sequence analysis. To address the question of their taxonomic position, amplified rDNA restriction analysis (ARDRA), DNA-DNA hybridization and phenotypic analysis were performed.

METHODS

Strain isolation. Samples from bulk vertisol were collected in the south-east of the island of Martinique in the French West Indies. The site has been under permanent grassland (*Digitaria decumbens*) vegetation.

EPS-producing bacteria were isolated from the 5–20 µm fraction on sugar-enriched media. Physical soil fractionation was done as described by Kabir *et al.* (1994). Several carbohydrates (glucose, sucrose, mannitol, sorbitol, xylose, mannose, arabinose and maltose) were used at high concentration (20 g l⁻¹) as carbon source for bacterial growth and EPS synthesis. The strains investigated in this study and their origin are listed in Table 1. The type strain of *B. graminis* C4D1M was provided by J. Balandreau (Viallard *et al.*, 1998).

Growth conditions and biochemical characterization. The strains were purified on trypticase soy agar (TSA; Difco). They were grown overnight on modified LB medium (l⁻¹: 10 g Tryptone, 5 g yeast extract, 5 g NaCl) at 28 °C and

phenotypically identified with BIOLOG GN microplates and Microlog release 3.5 software. To increase the number of biochemical characters, BIOLOG GP microplates for Gram-positive bacteria and API 20 NE strips (bioMérieux) were also used.

DNA amplification. Ten microlitres of water-washed suspension of cultures (OD₅₉₀ 0.5) were used for PCR amplification. *rrs* (16S rDNA) genes were amplified using a pair of universal primers (*fdI* and *rdI*) corresponding to positions 8–27 and 1524–1540, respectively, on the *Escherichia coli rrs* sequence. The reaction mixture containing bacterial cells was heated at 95 °C for 4 min before the addition of thermostable DNA polymerase. PCR consisted of 30 cycles (denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min). The final extension was at 72 °C for 3 min.

Amplified rDNA restriction analysis (ARDRA). The amplified *rrs* genes were analysed by restriction digestion with 12 restriction enzymes (*AluI*, *CfoI*, *DdeI*, *HinI*, *TaqI*, *HaeIII*, *MspI*, *RsaI*, *NdeII*, *NciI*, *Sau96I* and *ScrFI*). The restriction profiles were compared to those of the type strains of *B. cepacia* (ATCC 25416^T), *Burkholderia glathei* (LMG 14190^T), *B. vietnamiensis* (LMG 10929^T), *Burkholderia pyrrocinia* (LMG 14191^T), *Burkholderia caryophylli* (LMG 2155^T), *Burkholderia glumae* (LMG 2196^T), *Burkholderia gladioli* (LMG 2216^T), *Burkholderia plantarii* (LMG 9035^T), *Burkholderia cocovenenans* (LMG 11626^T), *Burkholderia andropogonis* (LMG 2129^T) and *B. graminis* (C4DIM^T).

PCR product direct sequencing. PCR products were purified on a 1% low-melting-point agarose gel and directly sequenced according to the protocol of Anderson *et al.* (1992). Seven primers were used in the sequencing reaction. These primers corresponded to the following positions in the *E. coli rrs* sequence: primer S3 (position 242–262), S6 (518–534), S8 (684–702), S10 (906–925), S12 (1099–1114), S15 (1384–1400) and S17 (1493–1509). Sequence reaction mixtures were subjected to electrophoresis on a 6% (w/v) polyacrylamide sequencing gel in a Beckman Genomix DNA sequencer.

Phylogenetic analysis. *rrs* sequences from strains MWAP64^T, MWAP71 and MWAP84 were aligned and analysed by comparison to a personal database of already aligned and classified sequences (about 7000). The new sequences were manually aligned over more and more divergent domains as successive phylogenetic analyses (neighbour-joining) assigned these sequences to more precise clades. When a definitive identification to *Burkholderia* was obtained, three phylogenetic methods were used to assess very precisely the exact phylogenetic position of these sequences: the neighbour-joining method of Saitou & Nei (1987), the maximum-likelihood method (options QFYG; EDNAML program of G. J. Olsen, University of Illinois, Urbana, IL, USA) and the maximum-parsimony method [PAUP program of D. Swoford (phylogenetic analysis using parsimony, version 3.0, distributed by the Illinois Natural History Survey, Champaign, IL, USA) for the Macintosh, heuristic search]]. The robustness of each topology was checked with the neighbour-joining method and 500 bootstrap replications using a Kimura two-parameter correction for computing distances. Trees were drawn with the NJPLOT program for the Macintosh (M. Gouy, CNRS UMR 5558, Université Claude Bernard, Lyon, France). Because all the sequences of strains shown in Fig. 1 are closely related, almost the entire sequences could be used to construct the dendrogram (positions 115–1376, *E. coli* numbering).

Table 1. Strains investigated in this study, their origin and *rrs* sequence accession number

Strain*	Origin/reference	GenBank no.
New isolates to be included in <i>B. caribensis</i>		
MWAP53	Vertisol (Martinique), this study	
MWAP54	Vertisol (Martinique), this study	
MWAP55	Vertisol (Martinique), this study	
MWAP62	Vertisol (Martinique), this study	
MWAP64 ^T (LMG 18531 ^T)	Vertisol (Martinique), this study	Y17009†
MWAP65	Vertisol (Martinique), this study	
MWAP67	Vertisol (Martinique), this study	
MWAP70	Vertisol (Martinique), this study	
MWAP71	Vertisol (Martinique), this study	Y17010†
MWAP74	Vertisol (Martinique), this study	
MWAP75	Vertisol (Martinique), this study	
MWAP76	Vertisol (Martinique), this study	
MWAP77	Vertisol (Martinique), this study	
MWAP78	Vertisol (Martinique), this study	
MWAP81	Vertisol (Martinique), this study	
MWAP82	Vertisol (Martinique), this study	
MWAP83	Vertisol (Martinique), this study	
MWAP84 (LMG 18532)	Vertisol (Martinique), this study	Y17011†
MWAP85	Vertisol (Martinique), this study	
MWAP86	Vertisol (Martinique), this study	
MWAP87	Vertisol (Martinique), this study	
Reference strains		
<i>Burkholderia</i> sp. LB400	–	U86373
<i>Burkholderia</i> sp. N3P2	Phenanthrene-enriched soil (Mueller <i>et al.</i> , 1997)	U37344
<i>Burkholderia</i> sp. N2P5	Phenanthrene-enriched soil (Mueller <i>et al.</i> , 1997)	U37342
<i>Burkholderia</i> sp. N2P6	Phenanthrene-enriched soil (Mueller <i>et al.</i> , 1997)	U37340
<i>Burkholderia</i> sp. CRE7	Phenanthrene-enriched soil (Mueller <i>et al.</i> , 1997)	U37343
<i>Burkholderia</i> sp. GSOY	–	U16140
<i>Burkholderia</i> sp. VUN100313	–	AF068011
<i>Burkholderia</i> sp. SAP II	–	AF052387
<i>B. glathei</i> LMG 14190 ^T	Germany (Zolg & Ottow, 1975)	Y17052†
<i>B. cepacia</i> ATCC 25416 ^T	<i>Allium cepa</i> (Ballard <i>et al.</i> , 1970)	U96927
<i>B. vietnamiensis</i> LMG 10929 ^T	Rice rhizosphere, Vietnam (Gillis <i>et al.</i> , 1995)	U96928
<i>B. pyrrocinia</i> LMG 14191 ^T	Unknown (Imanaka <i>et al.</i> , 1965)	U96930
<i>B. caryophylli</i> LMG 2155 ^T	<i>Dianthus caryophyllus</i> (Ballard <i>et al.</i> , 1970)	X67039
<i>B. glumae</i> LMG 2196 ^T	<i>Oryza sativa</i> , Japan	U96931
<i>B. gladioli</i> LMG 2216 ^T	<i>Gladiolus</i> sp., USA (Severini, 1913)	X67038
<i>B. plantarii</i> LMG 9035 ^T	<i>Oryza sativa</i> , Japan (Azegami <i>et al.</i> , 1987)	U96933
<i>B. cocovenenans</i> LMG 11626 ^T	Fermented coconut, Java (Vandamme <i>et al.</i> , 1960)	U96934
<i>B. andropogonis</i> LMG 2129 ^T	<i>Sorghum bicolor</i> (Stapp, 1935)	X67037
<i>B. phenazinium</i> LMG 2247 ^T	Soil (Bell & Turner, 1973)	U96936
<i>B. graminis</i> C4DIM ^T	Maize rhizosphere, France (Viallard <i>et al.</i> , 1998)	U96939
<i>Burkholderia</i> sp. m35b	Maize rhizosphere, France (Viallard <i>et al.</i> , 1998)	U96937
<i>Burkholderia</i> sp. C3B1M	Maize rhizosphere, France (Viallard <i>et al.</i> , 1998)	U96938
<i>B. thailandensis</i> E264	Environmental isolate (Brett <i>et al.</i> , 1997)	U91838
<i>B. pseudomallei</i> 1026b	Environmental isolate (Brett <i>et al.</i> , 1997)	U91839
<i>B. vandii</i> LMG 16020 ^T	Orchid rhizosphere (Urakami <i>et al.</i> , 1994)	U96932
<i>Burkholderia</i> sp. JB1	Garden soil (Springael <i>et al.</i> , 1996)	X92188

* ATCC, American Type Culture Collection, Manassas, VA, USA; LMG, Laboratorium Microbiologie Gent Culture Collection, Universiteit Gent, Gent, Belgium.

† Sequences determined in this study.

DNA–DNA hybridization. Genomic DNAs were extracted from the isolates and purified (Brenner *et al.*, 1982). DNA purity was monitored by measuring absorbance at 230, 260 and 280 nm (Genequant, Pharmacia). Genomic DNAs were sheared by sonication to produce a mean fragment size of 0.4 kb. DNA was labelled with [32 P]-dATP (Amersham) by nick translation with a commercial kit as recommended by Amersham. Labelled DNAs were prepared from strain MWAP64^T. DNA reassociation experiments were performed in triplicate in 300 μ l 0.42 M NaCl containing 75 μ g sheared, denatured unlabelled DNA and 0.07 μ g denatured labelled DNA, under stringent conditions (70 °C) according to the S1 nuclease–TCA method (Crosa *et al.*, 1973; Grimont *et al.*, 1980).

DNA base composition. The G+C content of strain MWAP71 was determined by the thermal denaturation method (Marmur & Doty, 1962) and was calculated by using the equation of Owen & Lapage (1976).

RESULTS AND DISCUSSION

Localization and phenotypic identification of the major EPS-producing MWAP strains

After fractionation of the vertisol, total cultivable bacteria were counted on tenfold-diluted TSA medium in different fraction sizes: < 5 μ m, 5–20 μ m, 20–50 μ m, 50–250 μ m and > 250 μ m. The 5–20 μ m fraction was described by Kabir *et al.* (1994) as the bacteria-enriched fraction in this soil. This was confirmed in this study and the cultivable bacteria population was estimated to be 5×10^7 c.f.u. (g dry soil)⁻¹ in the 5–20 μ m fraction. The 21 MWAP strains that were isolated from the 5–20 μ m fraction of the vertisol were identified as *B. cepacia* or as *B. glathei* by using BIOLOG Gram-negative microplates.

ARDRA

The *rrs* gene of the 21 MWAP strains and the 11 type strains of *Burkholderia* species were mapped with 12 restriction enzymes. The 21 MWAP strains provided exactly the same profiles with the tested restriction enzymes and belonged to a single ARDRA group. However, differences were observed with previously described species of *Burkholderia* (*B. cepacia* ATCC 25416^T, *B. glathei* LMG 14190^T, *B. vietnamiensis* LMG 10929^T, *B. pyrrocinia* LMG 14191^T, *B. caryophylli* LMG 2155^T, *B. glumae* LMG 2196^T, *B. plantarii* LMG 9035^T, *B. cocovenenans* LMG 11626^T, *B. andropogonis* LMG 2129^T, *Burkholderia phenazinium* LMG 2247^T and *B. graminis* C4DIM^T) and at least five different profiles corresponding to five restriction enzymes were obtained for the closest species *B. graminis*.

rrs sequences and phylogenetic analyses

To perform a phylogenetic identification, *rrs* sequences of strains MWAP64^T, MWAP71 and MWAP84 were analysed. The results of broad phylogenetic analyses clearly showed that these strains belonged to the phylum *Proteobacteria* of the eubacteria (data not

Fig. 1. Unrooted phylogenetic tree of *Burkholderia* 16S rDNA sequences. This topology was obtained using all known complete or almost complete sequences from *Burkholderia* strains (see Table 1). The topology shown was obtained by neighbour-joining, the percentage values indicating the results of a bootstrap analysis (500 replications, only values above 50% are indicated). Congruences with maximum-likelihood and maximum-parsimony analyses are shown by * ($P < 0.01$ for ML) and +, respectively.

Table 2. DNA–DNA reassociation values between vertisol microaggregate MWAP isolate DNA and DNA of *B. cepacia*, *B. glathei* and *B. graminis* type strains

Strain*	Percentage DNA relatedness with <i>B. caribensis</i> MWAP64†‡
MWAP64 ^T	100
MWAP84	105 ± 2
MWAP53	80 ± 1
MWAP71	74 ± 3
<i>B. glathei</i> LMG 14190 ^T	11 ± 7
<i>B. cepacia</i> ATCC 25416 ^T	2 ± 1
<i>B. graminis</i> C4DIM ^T	18 ± 13

* DNA used as template.

† DNA used as probe. DNA reassociation values are mean values ± SD obtained from two independent experiments.

shown) and, more precisely, to the β -subclass. More detailed analyses showed that they were included in the *Burkholderia* genus. Three phylogenetic methods were then used (neighbour-joining, maximum-likelihood and maximum-parsimony), each of which grouped all of the *Burkholderia* sequences in a monophyletic taxon. All three MWAP strains formed a robust monophyletic unit (Fig. 1). Although we did not perform extensive DNA–DNA hybridization with each recognized *Burkholderia* species, such analyses were done for the most closely related species (Table 2). MWAP strains can be considered as a new species, different from the other *Burkholderia* species for the following reasons: (i) they constitute a line of descent that cannot be grouped with any recognized species, (ii) they show *rrs* sequence divergence of more than 3% from any recognized species (Stackebrandt & Goebel, 1994) and (iii) they have a very low level of DNA–DNA similarity with type strains of phylogenetically related species. Phylogenetically, *B. caribensis* is included in a robust clade containing two recognized species (*B. phenazinium* and *B. graminis*) and several strains not yet recognized as species, among which are *Burkholderia* sp. strain LB400, a polychlorinated biphenyl-degrading strain (U86373), and *Burkholderia* sp. strains N3P2 (U37344) and N2P5 (U37342), phenanthrene-degrading bacteria (Mueller *et al.*, 1997). The robustness of the clade suggests that other strains of this cluster may have similar degrading properties. Finally, except for strains N2P5, SAP II (AF052387) and *B. caribensis* strains, not even two of the other sequences could be grouped in a robust subclade, suggesting that they may all be different species (Fig. 1).

DNA–DNA hybridization

Values for DNA–DNA reassociation data are shown in Table 2. High levels of DNA homology were detected between strains MWAP64^T, MWAP84, MWAP53 and MWAP71 (ranging from 74 to 105%).

In contrast, the DNA hybridization values between MWAP strains and the type strains of *B. cepacia*, *B. graminis* and *B. glathei* were low and ranged from 1 to 20% (Table 2).

Phenotypic and physiological characteristics of MWAP strains

The 21 MWAP strains were Gram-negative, pleiomorphic rods, 1–2 µm long and 0.5 µm in diameter. They formed smooth white colonies with regular edges when grown overnight on TSA medium at 28 °C and mucoid colonies due to EPS production on sugar-enriched media. No production of diffusible or fluorescent pigment on TSA and *Pseudomonas* F agar media was observed. On PCAT medium, these strains formed white colonies, smaller than those of *B. cepacia*. Optimal growth was observed at 28 °C.

Table 3 shows the biochemical characters tested on BIOLOG GN and GP microplates which differentiated MWAP strains from the following closely related *Burkholderia* species (as defined according to *rrs* sequencing and ARDRA data): *B. glathei*, *B. graminis*, *B. phenazinium*, *B. caryophylli* and *B. andropogonis*.

Conclusion

Besides the proposal of a new name, *Burkholderia caribensis* sp. nov., two major conclusions on habitat and activity of this species can be drawn: (i) because of the physical fractionation of soil samples and the selection of the 5–20 µm fraction for isolation steps, it can be concluded that strains of this species were present in the core of microaggregates, and (ii) it constituted the dominant cultivable population in this soil fraction as revealed on carbon-rich medium. Unlike most of the *Burkholderia* strains isolated from various environments, the *Burkholderia* strains described in this study were not isolated on PCAT medium. The main objective of this study was to isolate the dominant EPS-producing bacterial species and not especially *Burkholderia* strains. However, these strains were able to grow on PCAT medium as most of the *Burkholderia* species did. The capacity of each strain of this species to produce high amounts of EPS on carbon-rich medium was confirmed in pure cultures supplemented with various carbohydrates.

Several phenotypic characteristics differentiated the MWAP isolates from the most closely phylogenetically related *Burkholderia* species (*B. glathei*, *B. graminis*, *B. phenazinium*, *B. caryophylli* and *B. andropogonis*). The MWAP isolates were unable to oxidize malonic acid on BIOLOG GN microplates, in contrast to the five tested *Burkholderia* species. According to *rrs* sequence analysis, *B. graminis* was the most closely related species (97% similarity) and eight biochemical characters enabled differentiation of this species from MWAP strains: oxidation of sucrose, D-raffinose, α -keto-

Table 3. Biochemical characters that differentiate *B. glathei*, *B. andropogonis*, *B. caryophylli*, *B. phenazinium* and *B. graminis* from MWAP isolates

BIOLOG data of *B. glathei*, *B. andropogonis*, *B. caryophylli*, and *B. phenazinium* were provided by G. Bochner and J. Young from BIOLOG Inc. *n*, Number of strains; +, all strains positive; −, all strains negative; (+), 50–90% strains positive; (−), 8–25% strains positive; ND, not determined.

Characteristic	<i>B. caribensis</i> (<i>n</i> = 21)	<i>B. graminis</i> (C4DIM ⁺)	<i>B. phenazinium</i> (<i>n</i> = 3)	<i>B. caryophylli</i> (<i>n</i> = 12)	<i>B. andropogonis</i> (<i>n</i> = 16)	<i>B. glathei</i> (<i>n</i> = 16)
D-Trehalose	+	+	−	(+)	−	−
Sucrose	−	+	+	+	−	−
D-Raffinose	−	+	−	−	−	−
α-Ketovaleic acid	+	−	+	−	−	+
Malonic acid	−	+	(+)	+	+	+
N-Acetylmannosamine	+	−	ND	ND	ND	ND
Lactamide	+	−	ND	ND	ND	ND
AMP	+	−	ND	ND	ND	ND
Glycyl-L-aspartic acid	−	−	+	(+)	−	(+)
Glucoronamide	+	+	−	(−)	(+)	(+)
DL-Carnitine	+	+	−	−	−	(+)
L-Ornithine	(−)	+	(+)	(+)	−	(−)
Lactose	−	+	−	−	(−)	+

valeric-acid, *N*-acetylmannosamine, lactamide, AMP, L-ornithine and lactose (Table 3).

The data from *rrs* sequences, ARDRA and DNA–DNA hybridization revealed that these EPS-producing bacteria formed a monophyletic unit in the genus *Burkholderia*. *rrs* sequence divergence, DNA–DNA hybridization data and biochemical characters allow the description of a new species for which we propose the name *Burkholderia caribensis* sp. nov.

Description of *Burkholderia caribensis* sp. nov.

Burkholderia caribensis (ca.ri.ben'sis. M.L. adj. *caribensis* pertaining to the Caribbean Islands, named after the once-indigenous Caribe Indians, where the strains were isolated).

Cells are short rods (1–2 × 0.5 µm), Gram-negative, motile and pleiomorphic in actively growing cultures (LB medium). Cells produce large amounts of EPS when grown on sugar-enriched agar media (2% glucose, xylose, fructose, sorbitol, arabinose, xylose, mannitol or inositol). Colonies are about 3 mm in diameter after 24 h incubation on tenfold-diluted TSA medium at 28 °C. They are white, smooth and mucoid. No diffusible or fluorescent pigment was observed. Oxidase, catalase, urease, arginine dihydrolase and β-galactosidase are produced but protease is not produced. The following are oxidized: Tweens 40 and 80, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, D-fructose, L-fucose, D-galactose, α-D-glucose, *myo*-inositol, α-lactose, lactulose, maltose, D-mannitol, D-mannose, psicose, L-rhamnose, D-sorbitol,

D-trehalose, xylitol, methylpyruvate, monomethylsuccinate, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleic acid, DL-lactic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucoronamide, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-phenylalanine, L-proline, L-pyroglytamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 6-phosphate, *N*-acetyl-D-glucuronic acid, lactulose, palatinose, D-ribose, salicin, D-tagatose, D-xylose, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, pyruvic acid, *N*-acetyl-L-glutamic acid, adenosine, 2'-deoxyadenosine, AMP, fructose 6-phosphate, *N*-acetylglucosamine, gluconate, caprate, malate, citrate and phenylacetate. Arginine dihydrolase, urease and β-galactosidase are produced. The following are not oxidized: α-cyclodextrin, *N*-acetyl-D-galactosamine, cellobiose, *i*-erythritol, gentiobiose, α-lactose, melibiose, β-methyl-D-glucoside, D-raffinose, sucrose, turanose, malonic acid, glycyl-L-aspartic acid, inulin, amygdalin, arbutin, α-D-lactose, maltotriose, D-melezitose, 3-methylglucose, α-methyl-D-glucoside, β-methyl-D-mannoside, palatinose, stachyose, TMP, UMP, nitrate, tryptophan, esculin and adipate. The G+C content is 63.1 mol%. The type strain of *Burkholderia caribensis* is MWAP64^T which has been deposited in the BCCM/LMG Culture Collection as

LMG 18531^T. The characteristics of this strain are identical to those described above for the species.

ACKNOWLEDGEMENTS

This work was supported by a grant from ORSTOM, CNRS, INRA and CIRAD in a framework of 'Action Incitative InterInstitutionnelle' entitled 'Biofonctionnement des sols tropicaux et gestion durable des terres'. We gratefully acknowledge Dr L. Sutra for G+C content determination and Drs G. Bochner and J. Young from BIOLOG Inc. for providing BIOLOG data on *Burkholderia* species.

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