Burkholderia sordidicola sp. nov., isolated from the white-rot fungus *Phanerochaete sordida*

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Two bacterial strains associated with the white-rot fungus *Phanerochaete sordida* were subjected to taxonomic investigation. The isolates, designated KCTC 12081^T and KCTC 12082, were Gram-negative, non-motile, non-spore-forming and ovoid to rod-shaped. The strains contained major amounts of hexadecanoic acid, *cyclo*-heptadecanoic acid and ω-7-*cis*-octadecenoic acid in their cell envelopes. Strain KCTC 12081^T contained ubiquinone-8 as the major isoprenoid quinone and the G+C content of its genomic DNA was 61·3 mol%. Morphological and chemotaxonomic properties of the strains were consistent with classification in the genus *Burkholderia*. In a comparison of 16S rDNA sequence, KCTC 12081^T shared 100 % similarity with KCTC 12082 and both strains formed a distinct phylogenetic lineage within the genus *Burkholderia*. The two strains were also differentiated from other species of this genus by fatty acid composition and phenotypic properties. DNA–DNA relatedness data further supported the separation of the new isolates from closely related species. It is therefore proposed that strains KCTC 12081^T (=JCM 11778^T) and KCTC 12082 be recognized as a novel species, for which the name *Burkholderia sordidicola* sp. nov. is proposed.

Phanerochaete sordida is a widely distributed white-rot fungal species and is one of the most common corticioid fungi, typically inhabiting fallen branches of hardwood trees (Eriksson *et al.*, 1978; Lim *et al.*, 2000). White-rot fungi degrade lignin more extensively and rapidly than any other organism known (Kondo *et al.*, 1994; Garzillo *et al.*, 1998; Novotný *et al.*, 2000). *P. sordida* has been applied to a variety of industrial processes, such as biopulping and pulp bleaching, and also used in bioremediation, due to its enzymic capabilities (Lamar *et al.*, 1990).

A range of bacteria that belong to different subdivisions of the *Proteobacteria*, e.g. *Burkholderia fungorum*, have been found in association with the white-rot fungus *Phanerochaete chrysosporium* (Seigle-Murandi *et al.*, 1996; Coenye *et al.*, 2001a).

Burkholderia is a group of metabolically versatile Gram-negative bacteria. The genus was first proposed by Yabuuchi *et al.* (1992), who transferred seven species of rRNA group II of the genus *Pseudomonas* to the novel genus on the basis of a polyphasic taxonomic study. Members of the genus *Burkholderia* are often found in contaminated soil and water, as well as in natural soil, water and the rhizosphere of plants, and are capable of degrading numerous low-molecular-mass organic compounds including aromatic compounds, halogenated derivatives and various recalcitrant organic residues (Friedrich *et al.*, 2000; Nogales *et al.*, 2001; Parke & Gurian-Sherman, 2001).

In an isolation study of *P. sordida* strains collected from the plants *Quercus acutissima* and *Prunus serrulata*, bacterial strains were found to be associated with *P. sordida* cultures. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strains belonged to the genus *Burkholderia* and that they were apparently distinct from any known species in the genus. Detailed phenotypic and genotypic features of these bacterial isolates are presented in this study.

Strains KCTC 12081^T (=JCM 11778^T) and KCTC 12082 (=JCM 11779) were isolated from cultures of the white-rot fungi *P. sordida* KCTC 26213 (collected from *Quercus acutissima*, Kangwha, Inchon, Korea) and *P. sordida* KCTC 26214 (collected from *Prunus serrulata*, Seoul, Korea), respectively. Bacterial strains were subcultured on tryptic soy agar [tryptic soy broth (Difco) supplemented with 1·5 % agar] and grown aerobically at 30 °C. 16S rDNA was PCR-amplified by using primers 27f and 1492r (Lane, 1991). PCR products were purified by using an AccuPrep PCR Purification kit (Bioneer) and sequenced by using primers 27f, 803f, 907r and 1492r (Lane, 1991). Nearly complete 16S rRNA gene sequences (corresponding to positions 63–1453

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of the *Escherichia coli* numbering system) were obtained and aligned with those of 23 *Burkholderia* species with validly published names that were retrieved from GenBank (http://www.ncbi.nlm.nih.gov). Phylogenetic trees based on the neighbour-joining, Fitch–Margoliash and maximum-likelihood methods were inferred by using the PHYLIP package (Felsenstein, 1993). Distances for the first two algorithms were calculated by using the Jukes–Cantor distances option.

DNA–DNA relatedness was determined by using a DIG-High Prime DNA Labeling and Detection kit (Roche Applied Science) and Bio-Dot SF slot-blotting apparatus (Bio-Rad). Hybridization was performed at 42 °C; labelling of probes, blotting, hybridization and detection were carried out according to the manufacturers' instructions.

The molar G+C content of genomic DNA was determined by the thermal denaturation (T_m) method described by Marmur & Doty (1962). Major isoprenoid quinones were detected by using the method of Yamada (1998). The Biolog GN system was used according to the manufacturer's instructions to test the degradation of 95 carbon substrates for the two test strains and type strains of eight related species. All tests were run in duplicate.

Cultures of *P. sordida* KCTC 26213 and KCTC 26214 were always found to be associated with bacterial strains and it was not possible to obtain pure fungal cultures despite repeated trials. In contrast, the bacterial strains could be isolated easily from the mixed cultures and grew well on fungal culture media as well as on complex bacterial media, such as tryptic soy agar. A similar case on *P. chrysosporium* has been reported previously (Seigle-Murandi *et al.*, 1996; Coenye *et al.*, 2001a).

On agar plates, colonies of strains KCTC 12081^T and KCTC 12082 were cream to light ochraceous in colour. No diffusible pigment was produced. Under scanning electron microscopic observation, cells appeared as ovoid to short rod-shaped, approximately 1·3–1·7 μm in length and 1·1 μm in width (Fig. 1). Cells were non-motile, non-spore-forming and Gram-negative.

Strain KCTC 12081^T contained major amount of ubiquinone-8 (UQ-8) (approx. 95 % of total quinones) and also small amounts of UQ-7 (3 %) and UQ-9 (0·5 %). The genomic DNA G+C content of the strain, as determined by the T_m method, was 61·3 mol%. Both strains contained hexadecanoic acid ($C_{16:0}$), cyclo-heptadecanoic acid ($C_{17:0}$ cyclo) and ω-7-*cis*-octadecenoic acid ($C_{18:1}\omega7c$) as the major membrane fatty acid components, and also small amounts of 3-hydroxyhexadecanoic acid ($C_{16:0}$ 3-OH), the presence of which is characteristic for members of the genus *Burkholderia* (Table 1; Gillis *et al.*, 1995). Morphological and chemotaxonomic properties of the strains were consistent with their classification within the genus *Burkholderia* (Yabuuchi *et al.*, 1992; Gillis *et al.*, 1995).

The 16S rDNA sequences of strains KCTC 12081^T and KCTC 12082 were found to be identical. The strains were related to *Burkholderia glathei* ATCC 29195^T with 97·3 % similarity (corresponding to 38 nucleotide differences out of 1414 positions) and *Burkholderia phenazinium* LMG 2247^T with 97·2 % similarity (corresponding to 41/1438 differences). Mean similarity of the 16S rDNA sequence of the test strains against those of 23 *Burkholderia* species with validly published names was 91·6±0·7 %. The phylogenetic position of strains KCTC 12081^T and KCTC 12082 within the genus *Burkholderia* is shown in Fig. 2, in which the two strains formed an independent phylogenetic lineage that was related most closely to the clade that included *B. glathei* and *Burkholderia graminis*. The two test strains and *B. fungorum* Coenye *et al.* (2001a), another species known to be associated with a white-rot fungus (Siegle-Murandi *et al.*, 1996), were distantly related as 16S rDNA sequence similarity between the type strains was 96·1 % (57/1459 nucleotide differences). The relationship between

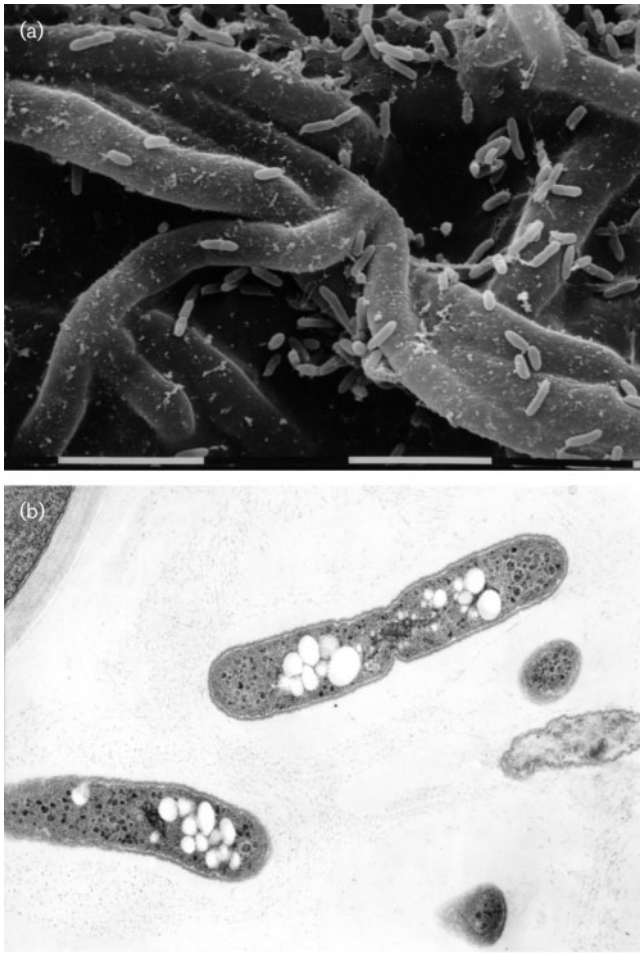

Fig. 1. Electron microscopic images of strain KCTC 12081^T. (a) Scanning electron microscopic image of KCTC 12081^T cells on the surface of fungal mycelia (bars, 10 μm); (b) thin-section image of KCTC 12081^T cells taken by transmission electron microscopy. Bright globules within the cell are cellular reserve materials.

Table 1. Fatty acid composition of selected strains

Strains: 1, KCTC 12081^T; 2, KCTC 12082; 3, *B. glathei* KCTC 2968^T. Numbers indicate percentage values of total amounts.

Fatty acid	1	2	3
$C_{12:0}$	3·3	3·04	–
$C_{14:0}$	0·6	0·59	4·2
Summed feature I*	3·6	3·07	4·9
Summed feature II†	10·2	7·45	10·5
$C_{16:0}$	20·9	17·49	20·2
$C_{17:0}$ cyclo	19·1	23·56	18·6
$C_{17:0}$	–	–	0·2
$C_{16:1}$ 2-OH	1·91	1·97	0·3
$C_{16:0}$ 2-OH	2·13	2·63	1·2
$C_{16:0}$ 3-OH	4·4	3·93	4·2
$C_{18:1}\omega7c$	24·3	23·28	30·5
$C_{18:0}$	0·76	0·69	0·6
$C_{19:0}\omega8c$ cyclo	8·4	11·14	4·5

*Composed of $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I.
†Composed of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

the test strains and related species in the neighbour-joining tree was not strongly supported by bootstrap analysis and the tree topology around the test strains was not recovered in all other trees generated by different algorithms (Fig. 1). However, the similarity level of 97·3 % between the test strains and *B. glathei* ATCC 29195^T is comparable to the values among the 13 species of the *Burkholderia cepacia* clade, which range between 96·1 and 99·9 % with a mean of 98·3 %.

DNA–DNA relatedness between strain KCTC 12081^T and *B. glathei* KCTC 2968^T was 13 % and that between KCTC 12081^T and *B. phenazinium* KCTC 2971^T was 15 %; both values are well below 70 %. These values are comparable to similar cases, for example, 11 % between *B. cepacia* LMG 1222^T and *Burkholderia gladioli* LMG 2216^T (sharing 98·2 % 16S rDNA sequence similarity) and 36 % between *B. cepacia* LMG 1222^T and *Burkholderia vietnamiensis* LMG 10929^T (sharing 99·4 % 16S rDNA sequence similarity) (Vandamme *et al.*, 1997). In another study, all species pairs that shared >98·1 % 16S rDNA sequence similarity resulted in DNA relatedness levels of <58 % (Coenye *et al.*, 2001b). Thus it is apparent by comparison of 16S rDNA sequence and DNA–DNA relatedness that strains KCTC 12081^T and KCTC 12082 are sufficiently distant from *B. glathei* ATCC 29195^T, *B. phenazinium* LMG 2247^T and other closely related species to be recognized as an independent taxospecies.

The test strains and *B. glathei* were also distinguished by comparison of fatty acid profiles and other phenotypic characteristics (Tables 1 and 2). One major fatty acid component, $C_{18:1}\omega7c$, exceeded 30 % of the total amount in *B. glathei*, whereas the same component comprised about

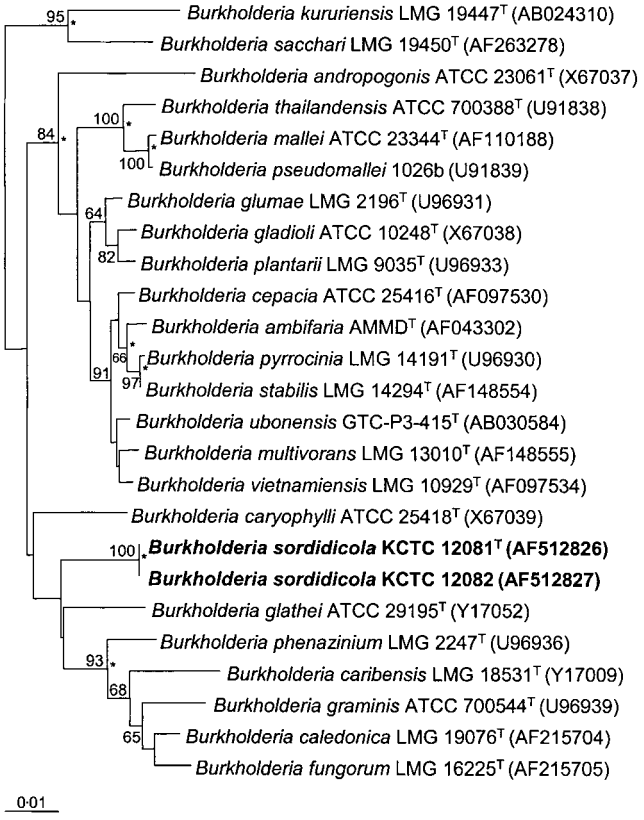

Fig. 2. Neighbour-joining tree based on 1300 unambiguously aligned positions of representative *Burkholderia* 16S rDNA sequences. Jukes–Cantor distances were used to construct the distance matrix. Numbers at nodes indicate levels of bootstrap support based on 1000 resamplings and asterisks indicate branches that were also recovered by using the Fitch–Margoliash and maximum-likelihood methods. Bar, 0·01 substitutions per nucleotide position.

23–24 % in strains KCTC 12081^T and KCTC 12082 (Table 1; Vandamme *et al.*, 1997). The two test strains and *B. glathei* can also be differentiated by motility, as the test strains are not motile but members of *B. glathei* are motile by a polar flagellum (Palleroni, 1984). The Biolog test proved helpful for species discrimination (Table 2). The test strains and closely related species were separated by at least 15 differences, with the exception of KCTC 12081^T and *B. phenazinium* KCTC 2971^T; these two strains differed in nine tests, but 32 tests out of 83 could not be compared because of the variable results for either strain (Table 2). There were 11 differences between strains KCTC 12081^T and KCTC 12082 (Table 2). Tests that showed identical results for all strains, namely 2,3-butanediol, α-D-glucose, L-glutamic acid, DL-lactic acid and methyl pyruvate (all positive) and cellobiose, i-erythritol, glycyl L-aspartic acid, α-ketovaleric acid, α-D-lactose, D-melibiose and thymidine (all negative) were excluded from Table 2.

It is evident that strains KCTC 12081^T and KCTC 12082 form a distinct centre of taxonomic variation within the

Table 2. Carbon source utilization of test strains and related *Burkholderia* species

Taxa: 1, KCTC 12081^T; 2, KCTC 12082; 3, *B. caribensis* KCTC 2964^T; 4, *B. caryophylli* KCTC 2965^T; 5, *B. cepacia* KCTC 2966^T; 6, *B. fungorum* KCTC 12917^T; 7, *B. glathei* KCTC 2968^T; 8, *B. glumae* KCTC 2969^T; 9, *B. phenazinium* KCTC 2971^T; 10, *B. vietnamiensis* KCTC 2974^T. +, Positive; −, negative; V, variable.

Compound	1	2	3	4	5	6	7	8	9	10
α-Cyclodextrin	−	−	−	−	−	V	−	−	−	−
Dextrin	−	−	−	−	−	+	−	−	−	−
Glycogen	−	−	−	−	−	−	−	−	V	−
Tween 40	V	+	+	+	+	+	+	+	+	+
Tween 80	V	+	+	+	+	+	+	+	V	+
N-Acetyl-D-galactosamine	−	−	V	−	−	−	+	−	−	−
N-Acetyl-D-glucosamine	+	+	+	+	+	−	+	+	+	+
Adonitol	+	+	+	−	V	−	+	+	+	−
L-Arabinose	−	V	+	+	+	−	+	+	−	+
D-Arabitol	+	+	+	+	+	−	+	+	V	+
D-Fructose	+	V	+	+	+	+	+	+	+	+
L-Fucose	+	+	+	+	+	−	+	+	+	−
D-Galactose	+	+	+	+	+	−	+	+	+	+
Gentiobiose	−	−	−	−	−	−	+	−	−	−
m-Inositol	V	−	+	+	+	−	+	+	−	+
Lactulose	+	−	+	−	+	−	−	−	−	−
Maltose	−	−	−	−	−	+	−	−	−	−
Mannitol	+	+	+	+	+	−	+	+	V	+
D-Mannose	+	−	+	V	+	+	+	+	V	+
Methyl β-D-glucoside	−	−	−	−	−	V	−	−	−	−
D-Psicose	V	−	−	−	−	+	−	−	V	−
D-Raffinose	−	−	−	−	−	−	−	−	−	+
L-Rhamnose	+	+	+	+	+	−	−	−	V	−
D-Sorbitol	+	+	+	+	+	−	+	+	V	+
Sucrose	−	−	−	+	−	−	+	−	−	+
D-Trehalose	−	−	+	+	−	+	+	+	−	+
Turanose	−	−	−	−	−	V	−	−	−	−
Xylitol	+	−	−	+	+	−	−	−	+	−
Monomethyl succinate	V	+	−	V	+	−	+	V	V	V
Acetic acid	V	−	V	−	V	V	V	V	V	V
cis-Aconitic acid	−	−	+	+	+	−	+	+	V	+
Citric acid	−	−	+	+	+	−	+	+	V	+
Formic acid	V	+	+	+	+	−	V	+	V	V
D-Galactonic acid lactone	−	+	+	V	+	−	+	V	V	+
D-Galacturonic acid	−	−	+	+	+	−	+	−	−	+
D-Gluconic acid	+	+	+	+	+	−	+	+	+	+
D-Glucosaminic acid	+	+	+	+	+	−	+	+	+	+
D-Glucuronic acid	+	−	+	+	+	−	+	−	−	+
α-Hydroxybutyric acid	V	V	+	V	+	−	V	−	+	V
β-Hydroxybutyric acid	+	+	+	+	+	−	+	+	+	+
γ-Hydroxybutyric acid	−	−	−	−	V	−	−	−	−	−
p-Hydroxyphenylacetic acid	−	−	+	+	+	−	+	−	+	V
Itaconic acid	−	−	−	−	+	−	−	−	−	+
α-Ketobutyric acid	V	V	+	−	+	+	+	V	+	−
α-Ketoglutaric acid	−	−	+	+	−	−	−	−	+	−
Malonic acid	V	−	V	V	+	−	+	+	−	+
Propionic acid	−	−	−	V	+	−	+	+	V	+
Quinic acid	−	+	+	+	+	−	+	+	−	+
D-Saccharic acid	−	+	+	+	+	−	+	+	−	+
Sebacic acid	V	+	−	−	V	−	+	V	V	+

Table 2. cont.

Compound	1	2	3	4	5	6	7	8	9	10
Succinic acid	+	+	+	V	+	−	+	+	+	+
Bromosuccinic acid	+	+	+	V	+	−	+	+	+	+
Succinamic acid	V	+	−	V	+	−	−	−	+	V
Glucuronamide	+	−	V	−	V	−	V	−	−	V
Alaninamide	V	−	+	V	V	−	−	V	+	−
D-Alanine	−	−	+	+	V	−	V	+	+	+
L-Alanine	V	+	+	+	V	−	+	+	+	+
L-Alanylglycine	+	−	+	+	V	−	+	+	+	+
L-Asparagine	+	+	+	+	+	V	+	+	+	+
L-Aspartic acid	+	V	+	+	+	−	+	+	+	+
Glycyl L-glutamic acid	V	−	+	−	−	−	−	−	+	−
L-Histidine	V	−	+	+	V	−	+	+	+	+
L-Hydroxyproline	−	−	+	+	+	−	+	+	−	+
L-Leucine	−	−	+	−	V	−	−	−	V	−
L-Ornithine	−	−	−	−	−	−	−	−	−	V
L-Phenylalanine	V	+	+	V	+	−	+	+	+	+
L-Proline	+	+	+	+	+	−	+	+	+	+
L-Pyroglutamic acid	V	−	+	+	+	−	+	−	+	+
D-Serine	−	−	−	−	−	−	+	−	−	−
L-Serine	V	V	+	+	+	−	+	+	+	+
L-Threonine	V	−	+	+	+	−	+	+	+	+
DL-Carnitine	V	−	+	−	−	−	V	+	−	V
γ-Aminobutyric acid	−	V	+	V	V	−	+	+	−	+
Urocanic acid	−	+	−	−	−	−	+	−	+	+
Inosine	−	−	+	−	−	V	−	−	−	−
Uridine	+	−	−	−	−	V	−	−	−	−
Phenylethylamine	−	−	−	−	+	−	V	V	−	V
Putrescine	−	−	−	−	−	+	+	−	−	V
2-Aminoethanol	−	−	V	−	+	−	+	+	−	V
Glycerol	V	−	+	V	−	−	+	+	+	+
DL-α-Glycerol phosphate	−	−	+	−	−	−	V	+	+	+
Glucose 1-phosphate	−	−	−	−	−	−	V	−	−	−
Glucose 6-phosphate	−	−	+	−	+	−	+	+	+	+

genus *Burkholderia*. The name *Burkholderia sordidicola* sp. nov. is proposed to accommodate the test strains, which were associated with the white-rot fungus *Phanerochaete sordida*.

Description of *Burkholderia sordidicola* sp. nov.

Burkholderia sordidicola (sor.di.di′co.la. N.L. n. *sordida* from *Phanerochaete sordida*, a species of white-rot fungus; L. suff. n. *-cola* inhabitant; N.L masc. n. *sordidicola* inhabitant of *Phanerochaete sordida*).

Gram-negative, non-motile, non-spore-forming, ovoid to short rod-shaped cells (approx. 1·3–1·7 μm in length and 1·1 μm in width). Contains major amounts of UQ-8 and small amounts of UQ-7 and UQ-9 in the cell envelope. Major fatty acid components are $C_{16:0}$, $C_{17:0}$ cyclo and $C_{18:1}\omega7c$ and also smaller amounts of a summed feature (composed of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$); $C_{19:0}\omega8c$ cyclo and $C_{16:0}$ 3-OH are also found. Utilizes a number of carbon compounds, as shown in Table 2. Molar G+C content of genomic DNA of the type strain is 61·3 mol% (T_m method).

Two strains have been reported to date, both of which were isolated in association with cultures of white-rot fungi that belonged to the species *Phanerochaete sordida*. Type strain is KCTC 12081^T (=JCM 11778^T). Reference strain is KCTC 12082.

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