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Burkholderia bryophila sp. nov. and *Burkholderia megapolitana* sp. nov., moss-associated species with antifungal and plant-growth-promoting properties

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A polyphasic taxonomic study including DNA–DNA reassociation experiments and an extensive biochemical characterization was performed on 14 *Burkholderia* isolates from moss gametophytes of nutrient-poor plant communities on the southern Baltic Sea coast in northern Germany. The strains were classified within two novel species, for which the names *Burkholderia bryophila* sp. nov. and *Burkholderia megapolitana* sp. nov. are proposed. The former species also includes isolates from grassland and agricultural soil collected in previous studies. Strains *Burkholderia bryophila* 1S18^T (=LMG 23644^T =CCUG 52993^T) and *Burkholderia megapolitana* A3^T (=LMG 23650^T =CCUG 53006^T) are the proposed type strains. They were isolated from *Sphagnum rubellum* and *Aulacomnium palustre*, respectively, growing in the 'Ribnitzer Großes Moor' nature reserve (Mecklenburg-Pommern, Germany). All moss isolates of both novel species showed antifungal activity against phytopathogens as well as plant-growth-promoting properties.

The genus *Burkholderia* comprises more then 40 different species which occupy a wide array of ecological niches (Vandamme *et al.*, 2007). Among others, they occur in soil and water, in the plant rhizosphere and endophytically in roots and shoots, but also in and on fungal mycelia. *Burkholderia* species are also well-known for their biological and metabolic properties, which can be exploited for biological control of fungal diseases in plants but also for bioremediation and plant-growth promotion (Govan *et al.*, 1996; Holmes *et al.*, 1998; Parke & Gurian-Sherman, 2001). Despite the importance of these bacteria in biotechnology, little is known about their occurrence and original function in natural ecosystems. Particularly in recent years, a growing number of *Burkholderia* strains and species with beneficial interactions with their host plants have been reported, e.g. N_2-fixing *Burkholderia* species like *Burkholderia vietnamiensis* (Gillis *et al.*, 1995), *Burkholderia tropica* (Reis *et al.*, 2004), and *Burkholderia silvatlantica* (Perin *et al.*, 2006), nodulating strains from different plants (Vandamme *et al.*, 2002; Chen *et al.*, 2006), endosymbionts of leaf galls of *Psychotria* plants (Van Oevelen *et al.*, 2002, 2004) and endophytes with high activities of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which is able to influence the ethylene level in plants (Sessitsch *et al.*, 2005).

Abbreviations: ACC, 1-aminocyclopropane 1-carboxylate; AHL, *N*-acylhomoserine lactone.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 23646 (=1A11), LMG 23648 (=A5), LMG 23644^T (=1S18^T) and LMG 23650^T (=A3^T) are respectively AM489499–AM489502.

Whole-cell protein profiles of the novel strains are available as supplementary material with the online version of this paper.

Opelt & Berg (2004) showed that *Burkholderia* strains form dominant populations on mosses and that these strains show high antagonistic potential against fungal pathogens. In particular, on the moss *Sphagnum*, which forms peat-bog ecosystems highly relevant for the world climate, they are dominant and important components of the associated microbial community (Opelt & Berg, 2004; Belova *et al.*, 2006). In the present study, we determined the molecular and physiological characteristics of *Burkholderia* isolates associated with three moss species.

The mosses were sampled in the 'Ribnitzer Großes Moor' nature reserve near Rostock (54° 18′ N 12° 16′ E) on the southern Baltic Sea coast in north-eastern Germany. The moss *Aulacomnium palustre* Hedw. (family Aulacomniaceae) grows on the edge of a non-calcareous mire behind the dunes in the *Sphagnum*-rich birch wood. The moss species *Sphagnum rubellum* Hedw. and *Sphagnum palustre* L. (family Sphagnaceae) were found in a more open part of the centre of the mire, together with common cottongrass (*Eriophorum angustifolium*), cranberries (*Vaccinium oxycoccus*) and cross-leaved heath (*Erica tetralix*) in a *Sphagnum–Eriophorum* mire plant community. These habitats were characterized by different abiotic conditions, especially soil pH (acid and strong acid), moisture (medium wet and wet) and nutrient content (medium poor and poor) described by Precker (2000). Gametophytes were placed in sterile Petri dishes and transported to the laboratory and then samples (5 g) were transferred to sterile Stomacher bags. To extract the moss-associated bacteria from the gametophytes, 45 ml sterile 0.85 % NaCl was added and samples were homogenized in a Stomacher laboratory blender for 60 s at high speed (BagMixer; Interscience). Strains plated on R2A were analysed for antifungal activity against fungal pathogens and active strains were identified (Opelt & Berg, 2004). A total of 40 *Burkholderia* isolates were investigated in detail, and the collection analysed in this work is a subset of these isolates (Table 1). Unless otherwise stated, isolates were grown routinely in nutrient broth II (Sifin) and stored in broth containing 15 % glycerol at –70 °C.

For whole-cell protein electrophoresis, strains were grown on nutrient agar (Oxoid CM3) 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 the Pearson product-moment correlation coefficient were performed using the GelCompar 4.2 software package (Applied Maths). Whole-cell protein electrophoresis divided the 14 moss isolates into two clusters of isolates with highly similar profiles (see Supplementary Fig. S1 in IJSEM Online). Isolates A1, A3^T and A10 formed one cluster, whereas the remaining isolates formed a second cluster. Comparison with our database, which includes whole-cell protein profiles of reference strains of all currently known *Burkholderia* species and many isolates deposited by colleagues worldwide, did not reveal close matches in protein profile similarity in the case of the former isolates, but demonstrated that three soil isolates, LMG 22946, R-23363 and R-20926, had profiles that were highly similar to those of the other group of strains (Supplementary Fig. S1). None of these profiles was similar to those of reference strains of established *Burkholderia* species (data not shown). Isolates LMG 22946 and R-23363 were obtained from permanent

Table 1. Bacterial strains investigated in this study

Strain	Source
***Burkholderia megapolitana* sp. nov.**	
A1 (=LMG 23652 =CCUG 53008)	*Aulacomnium palustre* (March 2002, Germany)
A3^T (=LMG 23650^T =CCUG 53006^T)	*Aulacomnium palustre* (March 2002, Germany)
A10 (=LMG 23651 =CCUG 53007)	*Aulacomnium palustre* (March 2002, Germany)
***Burkholderia bryophila* sp. nov.**	
A2 (=R-25738 =CCUG 53001)	*Aulacomnium palustre* (March 2002, Germany)
A5 (=LMG 23648 =CCUG 53000)	*Aulacomnium palustre* (March 2002, Germany)
A17 (=R-30312 =CCUG 53005)	*Aulacomnium palustre* (March 2002, Germany)
A20 (=R-25727 =CCUG 52999)	*Aulacomnium palustre* (March 2002, Germany)
A22 (=R-25743 =CCUG 53002)	*Aulacomnium palustre* (March 2002, Germany)
1A11 (=LMG 23646 =CCUG 52996)	*Aulacomnium palustre* (November 2001, Germany)
1A16 (=LMG 23647 =CCUG 52997)	*Aulacomnium palustre* (November 2001, Germany)
1S5 (=LMG 23645 =CCUG 52994)	*Sphagnum rubellum* (November 2001, Germany)
1S18^T (=LMG 23644^T =CCUG 52993^T)	*Sphagnum rubellum* (November 2001, Germany)
2Sp58 (=LMG 23649 =CCUG 53003)	*Sphagnum palustre* (October 2003, Germany)
2Sp83 (=R-30311 =CCUG 53004)	*Sphagnum palustre* (October 2003, Germany)
LMG 22946 (=RG3-14 =CCUG 52998)	Permanent grassland (September 2003, The Netherlands)
R-23363 (=RG47-5 =CCUG 52995)	Permanent grassland (September 2003, The Netherlands)
R-20926 (=S512)	Agricultural soil (UK)

grassland in the course of a study of agricultural management and diversity of *Burkholderia* species (Salles *et al.*, 2006) and isolate R-20926 was obtained from agricultural soil in the course of a study of the diversity of *Burkholderia* isolates from woodland rhizosphere environments (Richardson *et al.*, 2002).

DNA for 16S rRNA gene sequencing was prepared by heating one or two colonies at 95 °C for 15 min in 20 µl lysis buffer containing 0.25 % (w/v) SDS and 0.05 M NaOH. Following lysis, 180 µl distilled water was added to the lysate. 16S rRNA genes were amplified using oligonucleotide primers complementary to highly conserved regions of bacterial 16S rRNA genes. The forward primer was 5′-AGAGTTTGATCCTGGCTCAG-3′ (hybridizing at positions 8–27 according to the *Escherichia coli* numbering system) and the reverse primer was 5′-AAGGAGGTGATCCAGCCGCA-3′ (positions 1541–1522). PCR products were purified by using a NucleoFast 96 PCR clean-up kit (Macherey-Nagel). Sequencing reactions were performed by using a BigDye terminator cycle-sequencing kit (Applied Biosystems) and purified by using a Montage SEQ_{96} sequencing reaction clean-up kit (Millipore). Sequencing was performed by using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The eight sequencing primers used are listed in Coenye *et al.* (1999). Sequence assembly was performed using the program AutoAssembler (Applied Biosystems). Sequences were compared and aligned with sequences retrieved from GenBank using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were subsequently performed using the BioNumerics 4.5 software package (Applied Maths).

Nearly complete 16S rRNA gene sequences were determined for strain A3^T (representing the first protein electrophoretic cluster) and strains A5, 1S18^T and 1A11. The 16S rRNA gene sequences for strains LMG 22946 and R-20926 were available from previous studies (GenBank accession numbers AY949200 and AJ300688, respectively). All sequences were compared with 16S rRNA gene sequences of *Burkholderia* strains available in public databases. Strain A3^T exhibited the highest 16S rRNA gene similarity towards the type strains of *Burkholderia fungorum*, *Burkholderia phytofirmans* and *Burkholderia phenazinium* (98.6, 98.2 and 98.1 % similarity, respectively). Similarity values towards 16S rRNA gene sequences of other *Burkholderia* species were below 98 %. The 16S rRNA gene sequences of strains A5, 1S18^T, 1A11, LMG 22946 and R-20926 were more than 99 % similar to each other. The highest 16S rRNA gene similarity values of this group to other sequences (in the range 97–98 %) were subsequently calculated towards the type strains of *Burkholderia terricola*, *B. fungorum*, *Burkholderia caledonica*, *B. phytofirmans*, *Burkholderia graminis* and *B. phenazinium* and towards strain A3^T. Fig. 1 shows a 16S rRNA gene sequence-based phylogenetic tree including the isolates from the present study and their closest phylogenetic neighbours.

High-molecular-mass DNA was prepared as described by Pitcher *et al.* (1989) and DNA–DNA hybridizations were

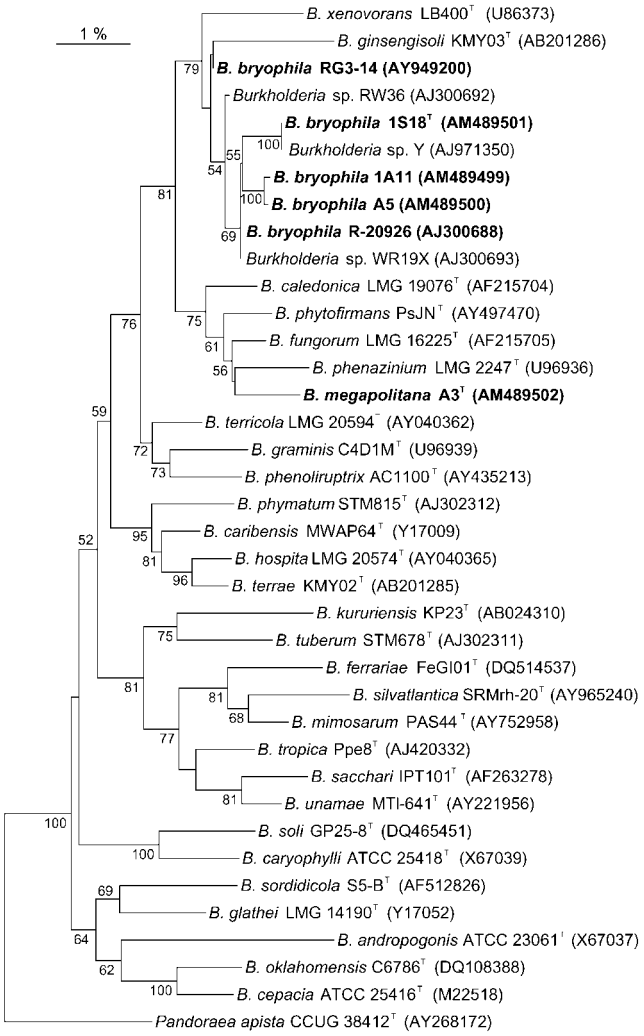

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strains of *B. megapolitana* sp. nov. and *B. bryophila* sp. nov. and their nearest phylogenetic neighbours within the genus *Burkholderia* based on 16S rRNA gene sequence comparisons. *Pandoraea apista* CCUG 38412^T was used as an outgroup in this analysis. Bar, 1 % sequence dissimilarity. Bootstrap values obtained with 1000 repetitions and higher than 50 % are shown as percentages. *Burkholderia* sp. strain Y was isolated by Belova *et al.* (2006) from a *Sphagnum* peat bog in Estonia; *Burkholderia* sp. strains RW36 and WR19X were isolated by Richardson *et al.* (2002) from rotting wood and woodland roots, respectively, in the UK.

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. Reciprocal experiments were performed for every pair of strains. Two sets of DNA–DNA hybridization experiments were performed. Genomic DNA of strain A3^T was hybridized with DNA of *B. fungorum* LMG 16225^T, *B. phytofirmans* LMG 22146^T and *B. phenazinium* LMG

2247^T; all values obtained were below 20 %. In a second series of hybridization experiments, genomic DNA of strains A5, 1S18^T and 1A11 was first hybridized with each other. All values obtained were above 75 %. Subsequently, genomic DNA of strain 1S18^T was hybridized with that of strain A3^T and with that of the type strains of its nearest phylogenetic neighbours, i.e. *B. terricola* LMG 20594^T, *B. fungorum* LMG 16225^T, *B. caledonica* LMG 19076^T, *B. phytofirmans* LMG 22146^T, *B. graminis* LMG 18924^T, *Burkholderia phenoliruptrix* LMG 22037^T and *Burkholderia xenovorans* LMG 21463^T; all these values were between 24 and 37 %.

DNA base ratios were determined as described by Mesbah *et al.* (1989). DNA was enzymically degraded into nucleosides and the nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C8 column thermostatted at 37 °C. The solvent was 0.02 M $NH_4H_2PO_4$ (pH 4.0) with 1.5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. The DNA G+C contents of strains A3^T (61.5 mol%), A5 (62.0 mol%), 1S18^T (62.5 mol%) and 1A11 (62.0 mol%) were very similar.

Classical phenotypic tests were performed as described previously (Vandamme *et al.*, 1993). API ZYM tests were performed according to the recommendations of the manufacturer (bioMérieux). Bacterial isolates were screened for their antagonistic activity and potential mechanisms of activity towards *Verticillium dahliae* V25, *Rhizoctonia solani* W4 and *Candida albicans* HRO1 (Strain Culture Collection, TU Graz) by a dual-culture *in vitro* assay on Waksman agar (WA) containing 5 g proteose-peptone (Merck), 10 g glucose (Merck), 3 g meat extract (Chemex), 5 g NaCl (Merck), 20 g agar (Difco) and distilled water (to 1 l), pH 6.8. Zones of inhibition were measured after 3 and 7 days of incubation at 20 °C. *In vitro* inhibition of *Erwinia carotovora* subsp. *atroseptica* NCPPB 549^T and *Xanthomonas campestris* DSM 3586 was determined in dual-culture assays on Luria–Bertani (LB) agar (Difco) in microtitre plates. From an overnight culture of bacteria, 10 µl was mixed with LB agar and bacterial isolates were spotted on the solidified agar surface. Zones of inhibition were measured after incubation at 20 °C for 24 and 48 h. All strains were tested in three independent replicates. Antagonistic activity against *Phytium ultimum* strain 67-1 (strain D261 from the collection of the phytopathology group of Professor Dr Geneviève Défago, ETH Zurich, Switzerland) was performed by dual-culture *in vitro* assay on potato dextrose agar (PDA) [containing 39 g PDA (Difco) consisting of 200 g infusion of potatoes, 20 g Bacto dextrose and 15 g Bacto agar per litre distilled water, pH 5.6]. Zones of inhibition were measured after 3–4 days of incubation at 20 °C.

Chitinase activity (β-1,4-glucosamine polymer degradation) was tested in chitin minimal medium according to Chernin *et al.* (1995). Clearing zones were detected 5 days after incubation at 20 °C. β-Glucanase activity was tested using chromogenic (azurine-dyed, cross-linked) AZCL substrates (Megazyme). Formation of blue haloes was recorded for 5 days of incubation at 20 °C. Protease activity (casein degradation) was determined from clearing zones in skimmed milk agar (50 ml sterilized skimmed milk mixed at 55 °C with 50 ml 1/5-strength TSA and 4 % agar) after 5 days of incubation at 20 °C.

Antibiosis against *Rhizoctonia solani* W4 by the bacterial strains was assayed on WA plates (15 ml) containing 5 ml sterile culture filtrate (64 h culture; nutrient broth II). The pH was adjusted to between 7 and 8. A 3 mm mycelial plug of *R. solani* W4 was placed in the centre of a WA plate. As a control, WA plates (20 ml) were similarly inoculated with mycelial plugs. Colony diameters were measured daily for 10 days and the reduction (%) in linear growth of the fungi was calculated. Siderophore production was assayed according to the method of Schwyn & Neilands (1987).

Production of *N*-acylhomoserine lactone (AHL) signal molecules was investigated with the aid of the GFP-based biosensor *Pseudomonas putida* F117 (pAS-C8) (Steidle *et al.*, 2001). This sensor is most sensitive for *N*-octanoylhomoserine lactone (C8-HSL), the most widespread AHL species synthesized by *Burkholderia* strains. AHL production was monitored in cross-streaking experiments on LB plates by the expression of GFP after illuminating plates with blue light.

ACC deaminase activity of the strains was tested on Brown & Dilworth (BD) minimal medium (Brown & Dilworth, 1975) containing 0.7 g ACC l^{-1} as a sole nitrogen source. BD plates containing 0.7 g NH_4Cl l^{-1} served as positive controls and BD plates containing no nitrogen source were used as negative controls. ACC deaminase production was monitored after 7 days of incubation at 30 °C.

The ability to promote plant growth was analysed in a microtitre assay using lettuce seeds. Surface-sterilized (1 % NaOCl, 5 min) lettuce seeds 'Daguan' (S 5601; Syngenta Seeds GmbH) were pre-germinated in moist chambers at 20 °C for 2 days. Sterility of seeds was proved by a print on nutrient agar. Three standard 24-well microplates (Roth) were filled with 1 ml water agar containing 12 g agar (Difco) made up to 1 l with distilled water (pH 6.8). One pre-germinated seed followed by 10 µl bacterial suspension (grown for 18 h in nutrient broth II) was added to each well after centrifugation and resuspension in physiological salt solution (0.85 %). In a preliminary experiment, different bacterial strains were evaluated at 10^3, 10^5, 10^7 and 10^9 c.f.u. ml^{-1} and compared with a control of 10 µl distilled water. A dose-dependent effect was found, with an optimal effect at a concentration of 10^5 c.f.u. ml^{-1}. Thus, bacterial isolates were evaluated at 10^5 c.f.u. ml^{-1} and compared with a control of 10 µl physiological salt solution (0.85 %). Two weeks after incubation (22/16 °C, 16/8 h day/night under artificial light) in a growth chamber (Percival Scientific), the number of leaves was counted and the length of the whole plant was measured to determine the effects of bacterial treatment on plant growth. Each strain was tested in 24 replicates.

Detailed overviews of the biochemical characteristics of the novel species are outlined below. Table 2 lists the results of analysis of the antagonistic activity of the moss-associated *Burkholderia* isolates. Table 3 presents biochemical characteristics that allow the novel species to be differentiated from their closest phylogenetic neighbours.

In conclusion, the data presented in this polyphasic taxonomic study demonstrate that the *Burkholderia* isolates examined represent two novel species that can be differentiated from their nearest phylogenetic neighbours by biochemical characteristics and whole-cell protein profiling. Below, we propose the name *Burkholderia megapolitana* sp. nov. to accommodate isolates A1, A3^T and A10 and the name *Burkholderia bryophila* sp. nov. to accommodate the remaining isolates. To our knowledge, the study of Opelt & Berg (2004) is the only one to have reported isolates of the former species. The known habitat of *B. megapolitana* is currently restricted to moss gametophytes in northern Germany. In contrast, Belova *et al.* (2006) recently reported that bacteria of the genus *Burkholderia* are a typical component of the microbial complex of *Sphagnum* peat bogs and constitute a substantial portion of the aerobic chemo-organotrophic isolates, which are routinely obtained from these environments on acidic nutrient media. They examined isolates from the peat of acidic *Sphagnum* bogs of the boreal and tundra zones of Russia, Canada and Estonia and obtained 16S rRNA gene sequences that were identical (or virtually identical) to those of our *B. bryophila* isolates (Fig. 1). The results of Belova *et al.* (2006) and our study suggest that *B. bryophila* is widely, but not exclusively, associated with moss gametophytes. In addition, the study of Richardson *et al.* (2002) included multiple isolates with 16S rRNA gene sequences virtually identical to those of strain R-20926 (=S512) and the other *B. bryophila* isolates. The additional strains were isolated from woodland roots and rotting wood (Fig. 1).

Description of *Burkholderia megapolitana* sp. nov.

Burkholderia megapolitana (me.ga.po.li.ta′na. L. fem. adj. *megapolitana* pertaining to Megapolis, the Latin name of Mecklenburg, the region from which the type strain was isolated).

Cells are Gram-negative, non-motile, non-sporulating, straight rods. Growth is observed at 28 °C. No acid production is observed in O/F medium with D-glucose, maltose, adonitol, D-fructose or D-xylose as carbon source. No production of fluorescent pigment. No catalase or oxidase activity. No growth on cetrimide agar (except for strain A3^T, which is weakly positive), on 10 % lactose

Table 2. Antagonistic activity and potential mechanisms of action of the moss-associated *Burkholderia* strains

Antagonism was assayed in dual-culture assays towards *Verticillium dahliae* (*V. d.*), *Rhizoctonia solani* (*R. s.*), *Erwinia carotovora* subsp. *atroseptica* (*E. c.*), *Candida albicans* (*C. a.*), *Staphylococcus aureus* (*S. a.*), *Xanthomonas campestris* (*X. c.*) and *Phytium ultimum* (*P. u.*) and the width of the inhibition zone is scored as follows: +, 0–5 mm; ++, 5–10 mm; +++, 10–15 mm. The presence of siderophores (++, >5 mm hydrolysis; +, 2–5 mm hydrolysis), activities of protease (Prot.) and β-glucanase (Gluc.) (+, hydrolysis; −, no hydrolysis) and ACC deaminase activity were determined in plate assays. Antibiosis was determined in an *in vitro* bioassay with sterile-filtered culture supernatant; results are percentages of fungal growth inhibition. AHL production was measured by cross-streaking against the GFP-based AHL biosensor *P. putida* F117 (pAS-C8). See text for further details. ND, Not determined.

Strain	Antagonistic activity against:							Siderophores	Lytic enzymes		ACC deaminase	Antibiosis (%)	AHL production
	V. d.	*R. s.*	*E. c.*	*C. a.*	*S. a.*	*X. c.*	*P. u.*		Prot.	Gluc.			
B. megapolitana													
A1	+	−	−	−	−	−	+	+	−	−	−	17.3	+
A3^T	++	+++	−	++	−	++	−	++	+	+	−	70.6	ND
A10	++	++	−	++	−	−	−	++	+	+	−	74.5	−
B. bryophila													
A2	+	++	−	−	−	−	+	++	−	−	+	76.5	−
A5	++	++	−	−	−	−	−	++	−	−	−	47.1	+
A17	+	+	−	−	−	−	+	+	−	−	−	42.3	−
A20	+	+	−	−	−	−	+	+	−	−	+	21.6	−
A22	++	−	−	−	−	−	+	+	−	−	−	49.0	+
1A11	+	−	−	−	−	−	+	++	+	−	+	77.4	−
1A16	+	−	−	−	−	−	+	++	+	−	+	77.4	−
1S5	+	−	−	−	−	−	+	+	−	−	+	19.6	+
1S18^T	+	−	+	−	−	+	−	+	−	−	+	21.2	−
2Sp58	+	++	−	−	+	−	+	+	−	−	−	42.6	−
2Sp83	++	+	−	−	++	+	ND	++	−	−	−	28.3	ND

Table 3. Differential biochemical characteristics between the novel strains and their nearest phylogenetic neighbours

Strains: 1, *B. megapolitana* sp. nov. (three strains); 2, *B. bryophila* sp. nov. (14 strains); 3, *B. caledonica* LMG 19076^T; 4, *B. fungorum* LMG 16225^T; 5, *B. graminis* LMG 18924^T; 6, *B. phenoliruptrix* LMG 22037^T; 7, *B. phytofirmans* LMG 22487^T; 8, *B. terricola* LMG 20594^T; 9, *B. xenovorans* LMG 21463^T. +, Positive; −, negative; W, weak or delayed reaction. Characteristics were determined for all *B. megapolitana* and *B. bryophila* strains included in the study.

Characteristic	1	2	3	4	5	6	7	8	9
Growth on blood agar at 37 °C	−	−	−	+	+	+	−	+	−
Growth on cetrimide agar	−	−	−	+	−	−	−	−	−
Acid production in O/F medium with:									
D-Glucose	−	−	+	+	+	W	−	+	W
Maltose	−	−	−	−	−	−	−	+	−
D-Xylose	−	−	W	W	W	−	−	−	+
Nitrate reduction	−	−	−	+	+	−	−	+	−
Assimilation of:									
Trehalose	+	−	W	−	W	−	−	W	−
Maltose	+	−	−	−	−	−	−	−	−
Citrate	−	+	−	+	W	W	−	W	+
Sucrose	+	−	−	−	+	−	−	+	−
Lactate	+	+	W	+	+	W	−	W	+

medium, in the presence of acetamide or in the presence of 0.5, 1.5, 3.0, 4.5 or 6.0 % NaCl. Grows on blood agar at 30 °C, but not on Drigalski agar, on blood agar at 37 °C or on nutrient agar at 42 °C. No reduction of nitrate or nitrite or denitrification. No liquefaction of gelatin or hydrolysis of aesculin. Tween 80 is hydrolysed. Haemolysis of horse blood is not observed. No production of acid or H_2S in triple-sugar-iron agar and no indole or pigment production. The type strain is susceptible to penicillin (10 μg per disc); the other known strains are resistant. β-Galactosidase (ONPG test), DNase, lysine and ornithine decarboxylase, arginine dihydrolase and urease (except for strain A10, which is weakly positive) activities are not detected. Assimilates D-glucose, trehalose, L-arginine, L-arabinose, D-mannose, D-mannitol, maltose, D-gluconate (weak for strain A1), caprate (weak for strain A1), L-malate, phenylacetate, sucrose, DL-lactate and DL-lactate with methionine, but not adipate or citrate. Assimilation of DL-norleucine is weak except in strain A3^T, which is clearly positive. Strong (strain A3^T) or weak (all other strains) assimilation of *N*-acetyl-D-glucosamine. Alkaline and acid phosphatase, leucine arylamidase and phosphoamidase activities are present. Activity of esterase C4, ester lipase C8 and chymotrypsin is weak. Amylase, lipase C14, valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are not detected. The DNA G+C content is about 61.5 mol%. So far strains have been only isolated from moss gametophytes in northern Germany. Plant-growth-promoting experiments demonstrate a clear effect on overall plant length (+31 %) and root length (+36 %) and primary leaf length (+57 %) (means of all strains in lettuce seedling assay in comparison with untreated controls). However, indole-3-acetic acid production determined according to Sarwar & Kremer (1995) is not observed.

The type strain is strain A3^T (=LMG 23650^T =CCUG 53006^T), isolated from *Aulacomnium palustre* at Graal-Müritz, Ribnitzer Großes Moor, in March 2002. Strains A1 (=LMG 23652 =CCUG 53008) and A10 (=LMG 23651 =CCUG 53007) are reference strains of this species and were isolated from the same source.

Description of *Burkholderia bryophila* sp. nov.

Burkholderia bryophila (bry.o.phi'la. Gr. n. *bryon* moss; Gr. adj. *philos* loving; N.L. fem. adj. *bryophila* moss-loving).

Cells are Gram-negative, non-motile (except for strain LMG 22946), non-sporulating, straight rods. Growth is observed at 28 °C. No acid production is observed in O/F medium with D-glucose (except for two isolates that yield weak growth), maltose, adonitol, D-fructose or D-xylose as carbon source. No production of fluorescent pigment. Catalase and oxidase activity are present (although some strains, including strain 1S18^T, give a weak reaction). No growth is observed on cetrimide agar (except for strain A20, which is weakly positive), on 10 % lactose medium, in the presence of acetamide or in the presence of 0.5 (except for strain LMG 22946), 1.5 (except for strain LMG 22946, which exhibits weak growth), 3.0, 4.5 or 6.0 % NaCl. Grows on blood agar at 30 °C but not on Drigalski agar (four strains, including strain 1S18^T, produce weak growth), on blood agar at 37 °C or on nutrient agar at 42 °C. No reduction of nitrate or nitrite or denitrification. No liquefaction of gelatin. Hydrolysis of aesculin is strain-dependent: only strain 1S18^T is clearly positive, two strains are weakly positive, while the others are negative. Tween 80 is hydrolysed (except for strain LMG 22946). Haemolysis of horse blood is not observed. No production of acid or H_2S in triple-sugar-iron agar and no indole or pigment production. Strains are resistant to penicillin (10 μg per disc) except for strain A5. β-Galactosidase activity (ONPG test) is present (except in strain A2). Lysine and ornithine decarboxylase, arginine dihydrolase, DNase and urease (except for two isolates that are weakly positive) activities are not detected. Assimilates D-glucose, L-arginine, L-arabinose (five isolates produce only weak growth), D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-gluconate (weak growth for two isolates), caprate (five isolates produce only weak growth), L-malate (except for one strain with a weak reaction), citrate (except for one strain with a weak reaction), phenylacetate (except for three strains with a weak reaction and one strain which is negative), DL-lactate (except for one strain with a weak reaction) and DL-lactate with methionine (except for one strain with a

weak reaction), but not trehalose (except for strain LMG 22946, which exhibits weak growth), DL-norleucine, maltose or sucrose. Assimilation of adipate is strain-dependent: two isolates including strain 1S18^T are negative, six give a weak reaction and the others are positive. Alkaline and acid phosphatase (except for strain LMG 22946, which exhibits weak reactions), leucine arylamidase and phosphoamidase (except for strain LMG 22946, which exhibits a weak reaction) activities are present. Activity of esterase C4 is strong in six strains and weak in the remaining isolates, including strain 1S18^T. Activity of ester lipase C8 is weak except for one strain which gives a strong reaction and one strain which is negative. Amylase (except in two strains where a weak reaction is observed), lipase C14 (except in one strain where a weak reaction is observed), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are not detected. The DNA G+C content is about 62 mol%. Strains have so far been isolated from moss gametophytes in northern Germany and from agricultural soil (UK) and grassland (The Netherlands). Plant-growth-promoting experiments demonstrate a clear effect on overall plant length (+55 %) and root length (+22 %) and primary leaf length (+50 %) (means of all strains in lettuce seedling assay in comparison with untreated controls). Indole-3-acetic acid production determined according to Sarwar & Kremer (1995) is not observed.

The type strain is strain 1S18^T (=LMG 23644^T =CCUG 52993^T), isolated from *Sphagnum rubellum* at Graal-Müritz, Ribnitzer Großes Moor, in November 2001. A number of reference strains of this species have been identified (Table 1).

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