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TAXONOMIC DESCRIPTION
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Paraburkholderia madseniana sp. nov., a phenolic acid-degrading bacterium isolated from acidic forest soil

Roland C. Wilhelm[1], Sean J. L. Murphy[1], Nicole M. Feriancek[1], David C. Karasz[1], Christopher M. DeRito[2], Jeffrey D. Newman[3] and Daniel H. Buckley[1,*]

Abstract

RP11^T was isolated from forest soil following enrichment with 4-hydroxybenzoic acid. Cells of RP11^T are aerobic, non-sporulating, exhibit swimming motility, and are rods (0.8 µm by 1.4 µm) that often occur as diplobacillus or in short chains (3–4 cells). Optimal growth on minimal media containing 4-hydroxybenzoic acid (µ=0.216 hr^{-1}) occurred at 30 °C, pH 6.5 or 7.0 and 0% salinity. Comparative chemotaxonomic, genomic and phylogenetic analyses revealed the isolate was distinct from its closest relative type strains identified as *Paraburkholderia aspalathi* LMG 27731^T, *Paraburkholderia fungorum* LMG 16225^T and *Paraburkholderia caffeinilytica* CF1^T. Strain RP11^T is genetically distinct from *P. aspalathi*, its closest relative, in terms of 16S rRNA gene sequence similarity (98.7%), genomic average nucleotide identity (94%) and *in silico* DNA–DNA hybridization (56.7%±2.8). The composition of fatty acids and substrate utilization pattern differentiated strain RP11^T from its closest relatives, including growth on phthalic acid. Strain RP11^T encoded the greatest number of aromatic degradation genes of all eleven closely related type strains and uniquely encoded a phthalic acid dioxygenase and paralog of the 3-hydroxybenzoate 4-monooxygenase. The only ubiquinone detected in strain RP11^T was Q-8, and the major cellular fatty acids were $C_{16:0}$, 3OH-$C_{16:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo ω8c, and summed feature 8 ($C_{18:1}$ ω7c/ω6c). On the basis of this polyphasic approach, it was determined that strain RP11^T represents a novel species from the genus *Paraburkholderia* for which the name *Paraburkholderia madseniana* sp. nov. is proposed. The type strain is RP11^T (=DSM 110123^T=LMG 31517^T).

INTRODUCTION

The genus *Paraburkholderia* was established from a division within the *Burkholderia* according to phylogenomic evidence [1]. The two genera were subsequently subdivided into four more genera, including *Trinickia*, *Caballeronia*, *Robbsia* and *Mycetohabitans* [2, 3]. Five *Paraburkholderia* type strains were transferred to the genus *Cabellaronia* (*P. glathei, P. grimmiae, P. humi, P. sordidicola* and *P. zhejiangensis*), three to *Trinickia* (*P. caryophylii*, *P. soli* and *P. symbiotica*), two to *Mycetohabitans* (*P. endofungorum* and *P. rhizoxinicia*) and one to *Robbsia* (*P. andropogonis*) [3, 4]. There are currently 64 type strains of *Paraburkholderia*, with 53 available genomes, of which the majority originate from soils (33 strains) or in associations with plant roots (23 strains), including rhizosphere, endophyte and root-nodulating species (Table S1, available in the online version of this article). Few strains have been described from aquatic environments with only a single freshwater [5], a single marine [6] and two root-associated aquatic type strains [7, 8]. Members of *Paraburkholderia* are of notable interest for their capacity to degrade aromatic compounds and the ability of some species to form root nodules that fix atmospheric N_2.

Paraburkholderia exhibit a range of metabolic capabilities, in part, due to their large genomes (7–10 Mb) and capacity to carry plasmids [9, 10]. *Paraburkholderia* have been described as facultative anaerobes [11], facultative chemolithotrophs [6, 12], acid-tolerant and alkalizing [7, 8, 13], metal-tolerant [6, 14], mineral weathering and phosphate solubilizing [14–16], polyaromatic hydrocarbon and xenobiotic degrading

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Keywords: *Paraburkholderia*; phenolic; 4-hydroxybenzoic acid; Burkholderiaceae; Forest soil.
Abbreviations: MSM, mineral salts medium; PHB, para-hydroxybenzoic acid=*p*-hydroxybenzoic acid=4-hydroxybenzoic acid; TSB, tryptic soy broth.
BioProject: PRJNA558488
Genome Accession: VOSW00000000
16S rRNA gene Accession: MN239497
NCBI Taxonomy ID: 2599607
Supplementary material is available with the online version of this article.

[10, 17], plant-growth promoting [18, 19] and nitrogen-fixing [6, 8, 20–24]. *Paraburkholderia* nodulate diverse temperate and tropical legumes (*Fabaceae*) [22, 25–33] or co-exist as root endophytes [18], increasing plant growth [34] and stress tolerance [35]. In soil, *Paraburkholderia* appear to play a role in the decomposition of plant-derived aromatics as evidenced by their capacity to degrade tannins and phenolics [36, 37], by their frequent isolation from acidic soils of forest and bogs [36, 38–44], and by their frequent isolation from wood colonized by lignin-degrading white-rot fungi [45, 46]. The capacity of soil isolates to degrade polycyclic aromatic hydrocarbons and halogenated phenols indicates a diverse role in degrading soil aromatics [11, 17, 47, 48]. In addition, aromatic- and phenolic acid-degrading *Paraburkholderia* have been implicated as principle contributors to the soil priming effect [49, 50].

ISOLATION AND ECOLOGY

Strain RP11^T (NCBI Taxonomy ID: 2599607) was isolated from the upper 1–5 mm of the A horizon of a Typic Fragiochrept soil (Inceptisol, pH 3.8–4.2) in an experimental forest (Dryden, NY. 42.450945,–76.420638) planted with red pine (*Pinus resinosa* Ait.). Its isolation was part of an effort to characterize phenolic acid-degrading populations involved in soil priming and was isolated by enrichment culturing with 4-hydroxybenzoate. Of several 4-hydroxybenzoate-degrading bacteria isolated, strain RP11^T was identified as a principle agent of priming [50]. After serial dilution, a soil slurry was spread plated onto mineral salts media containing 3 mM 4-hydroxybenzoate as the sole carbon source (MSM-PHB; recipe in Table S2). Colonies appeared after 3 days of growth at room temperature and strain RP11^T was streaked for isolation on MSM-PHB. For all subsequent chemotaxonomic characterizations, strain RP11^T was cultured on tryptic soy broth (TSB) at 30 °C and pH 7.0, while all characterizations of growth and morphology were performed with 25 mM MSM+PHB broth at 30 °C and pH 7.0 unless otherwise specified.

PHYLOGENETIC AND GENOME FEATURES

Genomic DNA from strain RP11^T was extracted according to the protocol of Griffiths *et al.* [51] and submitted to the Cornell University Sequencing Facility for sequencing using a single lane of Illumina MiSeq (2×250 bp). The genome assembly is available via the NCBI BioProject: PRJNA558488. Raw sequencing data was quality preprocessed with Trimmomatic (v. 0.32) [52] and FastX Toolkit (v. 0.7) [53] then assembled with SPAdes (v. 3.10.1) [54]. The assembly was comprised of 323 contigs, totaling 10 067 686 bases (N_{50}=84 334; avg. read depth=17×) and 9477 predicted open reading frames. Genomes from eleven of the closest related type strains of strain RP11^T (based on 16S rRNA gene homology) were downloaded from the National Centre for Biotechnological Information. Prodigal was used to predict open-reading frames (v. 2.6.2) [55] and oxidative genes were annotated using hmmsearch [56] with custom hidden-Markov models for laccases, aryl alcohol oxidases and dye-decoloring peroxidases developed by Wilhelm *et al.* [57]. Genomes were annotated using RAST [58] and grouped based on functional gene content in KBase [59] or phylogenetic relatedness using the BLAST Distance Phylogeny method implemented by the TYGS pipeline [60]. A phylogenetic tree was constructed with full-length 16S rRNA genes also based on BLAST Distance Phylogeny implemented in TYGS. *Cupriavidus necator* N-1^T, from the family *Burkholderiacaea*, served as the outgroup. Genome G+C content and the DNA–DNA hybridization values were predicted *in silico* based on genomic data [61]. The number of chromosomes, according to the number of origins of replication present, and copies of the *rrn* operon in strain RP11^T were determined based on the relative depth of unassembled sequencing reads mapping to annotated *oriC* and 16S rRNA genes versus single-copy genes identified using BUSCO [62].

The most closely related type strain to strain RP11^T was *P. aspalathi* based on whole-genome phylogeny (Fig. 1a), genomic average nucleotide identity, DNA–DNA hybridization and functional gene content (Table 1 and Fig. S1). The two strains also possessed the largest genomes and the lowest G+C content of all eleven closest relatives (Table 1). The values for average nucleotide identity and DNA–DNA hybridization between the two strains were below the respective thresholds (95 and 70%, respectively) for delineating new species [63, 64]. The phylogenetic relationships among type strains based on the 16S rRNA gene was inconsistent with the whole genome phylogeny and had low bootstrapped branch support, suggesting low resolution for this marker (Fig. 1b). The low discriminating power of 16S rRNA gene-based phylogenies for *Paraburkholderia* has previously been reported [13]. The genome of strain RP11^T encoded six copies of the *rrn* operon and two origins of replication homologous to *oriC* present on chromosome 1 and 2 of *P. caffeinilytica*.

The potential to metabolize aromatic compounds differentiated strain RP11^T from its closest relatives, with its genome encoding the greatest number of genes in the RAST SEED subsystem category for the metabolism of aromatics (Table 1) and the greatest number of aromatic-degrading oxidases (Table S3). The genomes of both strain RP11^T and *P. aspalathi* encoded a high number of oxidative enzymes compared to their closest relatives, including the same array of aryl alcohol oxidases, laccases and a DyP-type peroxidase (Table S3). However, only strain RP11^T encoded a phthalate 4,5-dioxygenase (EC 1.14.12.7) and was capable of growth on phthalic acid (2.5 mM) as a sole carbon source. All *Paraburkholderia* strains encoded at least one 3-hydroxybenzoate 4-monooxygenase gene (*pobA*; EC 1.14.13.23) consistent with the capacity for all tested strains to grow on 4-hydroxybenzoic acid. Only strain RP11^T, and the distantly related *P. megapolitana*, encoded a second paralogous copy of *pobA*. Each of RP11^T's paralogs were homologous to a *pobA* whose structure and function have been determined, namely *Pseudomonas* sp. CBS3 (67% identity) and *P. fluorescens* (76%), with shared identities at the key substrate binding and active site residues described by [65–67]. Strain RP11^T encoded several genes in

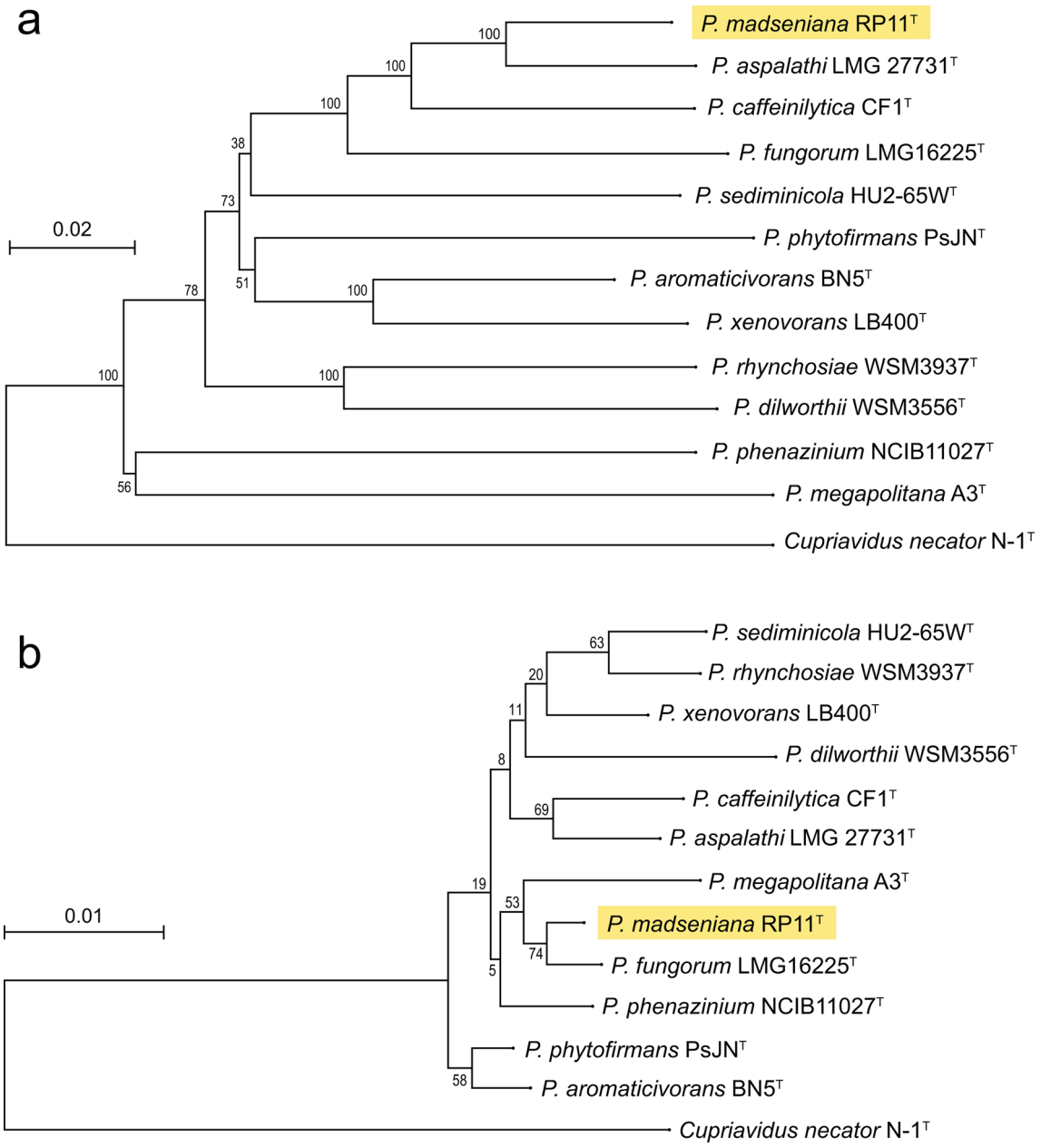

Fig. 1. The phylogenetic relationships of strain RP11^T with closely related species of *Paraburkholderia* according to a whole-genome phylogenetic tree based on BLAST Distance Phylogeny method using (a) genomes and (b) full-length 16S rRNA gene. Branch support correspond to pseudo-bootstrap values [60]. The scale bar corresponds to substitutions per nucleotide position. Accessions for genome assemblies and full-length 16S rRNA genes, respectively: *P. aspalathi* (GCF_900116445.1); *P. caffeinilytica* (GCF_003368325.1, NR_152088.1); *P. fungorum* (GCF_000685055.1, NR 025058.1); *P. aromaticivorans* (GCF_002278075.1, NR_163658.1); *P. phytofirmans* (GCF_000020125.1, NR_102845.1); *P. sediminicola* (GCF_900104005.1, NR_044383.1); *P. xenovorans* (GCF_000013645.1, NR_074325.2); *P. rhynchosiae* (GCF_002879865.1, NR_116248.1); *P. dilworthii* (GCF_000472525.1, NR_125580.1); *P. phenazinium* (GCF_900100735.1), *P. pallidirosea* DHOK13^T (NR_152705.1); *P. megapolitana* (GCF_900113825.1; NR_042594.1) and *Cupriavidus necator* (GCF_000219215.1, AF191737.1). If an accession is not specified, the 16S rRNA gene was recovered from the genome assembly indicated.

the oxygen-independent, beta-oxidation pathway for aromatic ring-cleavage (Fig. S2), also present in *P. aromaticivorans*, but neither genome encoded the complete pathway, nor did strain RP11^T exhibit anaerobic growth under any test condition.

Neither strain RP11^T nor its three closest relatives encoded the nitrogenase iron protein gene (*nifH*), which was present in *P. xenovorans, P. aromaticivorans* and *P. rhynchosiae*.

Table 1. Phylogenetic and genomic characteristics that differentiate strain RP11^T from its eleven most closely related type strains. Bolded values indicate closest matches to strain RP11^T. Columns were ordered by average nucleotide identity

Strains: 1, *P. madseniana* sp. nov. RP11^T; 2, *P. aspalathi* LMG 27731^T; 3, *P. caffeinilytica* CF1^T; 4, *P. fungorum* LMG 16225^T; 5, *P. aromaticivorans* BN5^T; 6, *P. phytofirmans* PsJN^T; 7, *P. sediminicola* HU2-65W^T; 8, *P. xenovorans* LB400^T; 9, *P. rhynchosiae* WSM3937^T; 10, *P. dilworthii* WSM3556^T; 11, *P. phenazinium* NCIB11027^T; 12, *P. megapolitana* A3^T.

	1	2	3	4	5	6	7	8	9	10	11	12
Genome size (Mb)	10.1	**9.89**	8.32	8.7	8.91	8.21	7.31	9.73	8.03	7.68	8.6	7.61
Number of contigs	385	104	3	124	8	3	118	3	**169**	141	56	32
G+C content (%)	61.3	**61.1**	62.2	61.8	62.9	62.3	63.6	62.6	61.7	61.8	62.3	62.1
Average nucleotide identity (%)	100	**94**	91.6	89.3	86.7	86.7	86.7	86.5	84.9	84.6	83.1	81.8
DNA–DNA hybridization	100	**56.7**	44.9	36.9	31	28.6	31.3	31.8	28.4	27.8	25.8	23.8
16S rRNA gene similarity (%)	100	98.7	98.4	**99.4**	98.8	99.1	98.6	98.6	98.5	98.1	97.9	98.5
Aromatic degradation genes*	191	**186**	158	158	185	133	104	186	159	136	122	133
Nitrogenase iron protein (*nifH*)	–	–	–	–	+	–	–	+	+	–	–	–
Cellulose synthase operon	+	+	+	+	+	+	–	+	+	+	+	–

*Total RAST SEED subsystem feature counts.

PHYSIOLOGY AND CHEMOTAXONOMY

Physiological and chemotaxonomic characterizations were performed for strain RP11^T and its three closest relatives, *Paraburkholderia aspalathi* LMG 27731^T, *Paraburkholderia fungorum* LMG 16225^T and *Paraburkholderia caffeinilytica* CF1^T. Determinations of enzyme activity and oxidation of carbon sources were performed using plate-based, colorimetric assays: API ZYM strips (bioMérieux, France) and Biolog GEN III plates (Biolog, USA), respectively, according to manufacturers' instructions. The capacity for growth on phenolic compounds was assessed in MSM media with 2.5 mM concentrations of benzoic acid, guaiacol, vanillin, syringic acid, ferulic acid, phthalic acid, salicylic acid and 0.3 mM 4-coumaric acid, due to low solubility. Growth was assessed according to differences in optical density (λ=600 nm) versus uninoculated controls. Cellular fatty acids were extracted from cells grown on nutrient agar for two days at 30 °C and methylated according to [68] using an Agilent 6850 gas chromatograph configured by Microbial ID Inc. (MIDI) with the Sherlock Microbial Identification System (v6.1) and the RTSBA6 database. Major respiratory quinones were determined by analysis of acetone extracts on an Agilent 6545 LC/Q-TOF MS using a modification of the methods described by [69] (details in Supplementary Methods). Oxidase activity was tested using Oxistrips (MilliporeSigma). Catalase activity was assessed based on the production of bubbles after mixing a drop of 3% H_2O_2 (v/v) (Wards Scientific) with a loop full of active culture. Gram-staining was performed according to the method of Smibert and Krieg [70]. Cell morphology and Gram-stain phenotype were observed by light microscopy (Olympus CX41).

For characterization of growth optima, RP11^T was cultivated aerobically in filter sterilized (0.22 μm) mineral salts medium (MSM) with 10 mM PHB or D-glucose as the sole carbon source. Prior to completion of final volume, the medium was brought to a pH of 7 using 1M HCl. Prior to growth assessments, all strains were passaged at least three times in MSM+PHB at pH 7. For each assay, cultivation of RP11^T was performed in triplicate in 20 ml test tubes bearing 10 ml MSM incubated at a slant for aeration and shaken at 180 r.p.m. on an orbital shaker. For determination of pH optima, MSM+PHB was prepared at pH 3, 4, 5, 6, 6.5, 7, 8 and 9 using buffer systems described in the Supplementary Methods. For determination of salinity optima, a concentrated stock solution of 5M NaCl was used to bring the NaCl concentration of the MSM+PHB broth to approximately 0.5, 1, 2 and 3% w/v in MSM+PHB at pH 7. For determination of growth temperature optima, RP11^T was cultivated at 5, 23, 30 and 37 °C in test tubes. Cell morphology was determined at a magnification of 1600× using a Zeiss Axioskop 2 and the program Axiovision v4.6.3. Cell dimensions were measured with the Axiovision length tool for 10 individuals at stationary phase.

The capacity for anaerobic growth on PHB or nutrient-rich media was tested in Balch tubes using 20 mm chlorobutyl stoppers and aluminum crimp seals. For nutrient-rich media, 5 ml aliquots of freshly prepared tryptic soy broth (DSMZ medium 92) and nutrient broth (DSMZ medium 1) were degassed using 20 cycles of vacuum and ultra-high purity N_2 gas (AirGas, PA), sealed and autoclave sterilized for 20 min. For minimal media tests, 5 ml of filter sterilized MSM with or without 25 mM PHB was added to pre-sterilized Balch tubes and degassed as above under sterile conditions. All anaerobic tests used an inoculum (2% v/v) of actively growing aerobic cultures. Anaerobic respiration was also tested using 20 mM glucose, 20 mM acetate or 25 mM PHB in MSM with 20 mM sodium nitrate as the terminal electron acceptor.

Strain RP11^T cells were Gram-negative and rod-shaped (0.8±0.1 µm by 1.4±0.2 µm), non-sporulating and oxidase- and catalase-positive. Cells grew as rods, occurring primarily as diplobacillus, but also individually or in short chains (3–4 cells). Cells were predominantly non-motile with increased proportions of cells exhibiting swimming motility during stationary phase. Motility was observed in all three type strains tested, contrary to the previous characterization of *P. caffeinlytica* as non-motile [11]. Strain RP11^T did not produce a visible biofilm like strain *P. fungorum* which formed a surface biofilm and flocculated during growth in TSB. The metabolic fingerprint for strain RP11^T was unique, and most closely resembled that of *P. aspalathi*, its nearest phylogenetic neighbour. Strain RP11^T was unique in metabolizing pectin, sucrose and raffinose and not inosine and myoinositol (Table 2). Strain RP11^T uniquely used phthalic acid as a sole carbon source for growth. Strain RP11^T exhibited enzyme activity characteristic of related strains, including acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and napathol-AS-BI-phosphohydrolase activities. The major fatty acids in strain RP11^T were $C_{16:0}$, 3OH-$C_{16:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo ω8c, and summed feature 8 ($C_{18:1}$ω7c and/or $C_{18:1}$ω6c). The abundances of $C_{16:0}$, $C_{17:0}$ cyclo and summed feature 8 discriminated strain RP11^T from related species (Table 3). The sole respiratory quinone observed in strain RP11^T was ubiquinone Q-8, which was consistent with reports for all related species [11, 45, 71].

The optimal growth rate for strain RP11^T was observed at 30 °C, pH 6.5–7.0 and 0% NaCl. In optimum conditions, strain RP11^T reached a specific growth rate 0.216 hr^{-1} that was comparable to its closest relative *P. aspalathi*, but higher than both *P. fungorum* and *P. caffeinilytica* (Fig. 2 and Table S4). Growth of RP11^T was diminished when growth media contained 0.5% NaCl and no growth was observed above 0.5%. RP11^T showed minimal growth at 37 °C and slower growth at 5 °C, reaching peak cell density in approximately 120 h. Aeration was important for growth on MSM+PHB, likely due to the oxygen-dependent enzymes involved in PHB catabolism. Strain RP11^T exhibited an alkalinizing effect, raising the pH of MSM+PHB (25 mM) from 7.0 to 8.15 by stationary phase. This effect was previously reported for *Paraburkholderia bannensis* [13].

DISCUSSION

Results from our polyphasic approach established that strain RP11^T constitutes a novel species in the genus *Paraburkholderia*. Strain RP11^T met all phylogenetic and chemotaxonomic criteria for a new species and exhibited notable functional differences to closely related strains. The name *Paraburkholderia madseniana* sp. nov. is proposed.

P. madseniana most closely resembled *P. aspalathi* in all genomic and functional comparisons, and *P. fungorum* in 16S rRNA gene similarity, though the latter indicator is not highly discriminatory for species of *Paraburkholderia* [1, 13]. The similarity between *P. madseniana*, a forest soil isolate,

Table 2. A summary of phenotypic characteristics that differentiate strain RP11^T from closely related species in the genus *Paraburkholderia*. All data are from this study

Strains: 1, *P. madseniana* sp. nov. RP11^T; 2, *P.aspalathi* LMG 27731^T; 3, *P. fungorum* LMG16225^T; 4, *P. caffeinilytica* CF1^T.

	1	2	3	4
BIOLOG				
D-Raffinose	+	-	-	-
Pectin	+	-	-	-
Sucrose	+	-	-	-
D-Saccharic acid	-	+	+	+
Inosine	-	+	+	+
Myo-inositol	-	+	+	+
α-Keto-glutaric acid	-	+	-	-
Troleandomycin*	-	+	-	-
Glycyl-L-proline	+	+	-	-
D-Malic acid	+	+	-	-
N-acetyl-D-galactosamine	-	-	+	+
L-Galacturonic acid lactone	+	+	+	-
D-Glucuronic acid	+	+	+	-
L-Serine	+	+	+	-
L-Lactic acid	+	+	+	-
α-Hydroxy-butyric acid	+	+	+	-
Acetic acid	+	+	+	-
D-Fucose	-	-	-	+
α-Keto-butyric acid	+	+	-	+
α-D-Glucose	-	-	+	-
D-Glucose-6-PO4	-	+	-	+
D-Fructose-6-PO4	-	+	-	+
D-Aspartic acid	+	+	-	+
D-Galacturonic acid	-	+	+	-
D-Fructose	+	-	+	+
Growth on phenolic acids				
4-Hydroxybenzoic acid	+	+	+	+
Benzoic acid	+	+	+	+
4-Coumaric acid	+	+	+	+
Phthalic acid	+	–	–	–
Ferulic acid	–	–	–	+
Vanillin	–	–	–	+
Syringic acid	–	–	–	–
Salicylic acid	–	–	–	–
Guaiacol	–	–	–	–

*Tolerance to antibiotic,

Table 3. Cellular fatty acid compositions of strain RP11^T and closely related species from the genus *Paraburkholderia*. Values are percentages of total fatty acids. Fatty acids that make up <1% of the total are not shown or are denoted as trace 'TR'. Bolded values indicate unique properties of strain RP11^T. All data are from this study.

Strains: 1, *P. madseniana* sp. nov. RP11^T; 2, *P.aspalathi* LMG 27731^T; 3, *P. fungorum* LMG16225^T; 4, *P. caffeinilytica* CF1^T.

	1	2	3	4
C12:0	2.7	2.56	TR	2.66
C14:0	1.35	1.5	5.64	1.36
C16:0	**32.65**	23.75	20.47	24.2
C16:1 2-OH	1.07	1.58	2.04	2.06
C16:0 2-OH	TR	3.61	2.66	2.35
C16:0 3-OH	4.59	4.27	4.95	4.28
C17:0 cyclo	**33.16**	25.06	19.03	21.95
C18:1 2-OH	TR	TR	1.34	1.04
C19:0 cyclo ω8c	9.54	11.94	13.81	9.23
Summed feature 2	5.23	4.89	6.03	5.01
Summed feature 3	1.2	2.34	2.57	4.93
Summed feature 8	**2.19**	14.1	16.06	16.8
Total %	93.68	95.6	94.6	95.87

and *P. aspalathi*, isolated from a root nodule, suggests a degree of shared ecology between these related species. High levels of plant-derived phenolic acids are common in both habitats, with 4-hydroxybenoic acid occurring at comparable concentrations in litter and plant roots [72]. Phenolic acids act as strong chemoattractants for root nodulating bacteria [73] and broadly facilitate plant-microbe interactions, though evidence for this in *Paraburkholderia* is lacking [74]. The unique capacity of *P. madseniana* to degrade phthalic acid supports its role in decomposition, since phthalic acids are common by-products of lignin-degradation [75–77] and phthalate dioxygenase and phthalate transporter genes were among several to confer high-levels of fitness to soil bacteria [78, 79]. *P. madseniana* was shown to prime the degradation of soil organic matter [49, 50], raising speculation about the role of its oxidative enzymes in carbon cycling.

Defining the ecology of *Paraburkholderia* is made challenging by historical and persistent misclassifications in literature pertaining to the genus. *Paraburkholderia* are commonly misidentified as *Burkholderia* or indeterminately categorized as part of the '*Burkholderia-Caballeronia-Paraburkholderia*' group [80]. Several studies of lignin or litter degradation and root- or fungi-associated bacteria report major trends in '*Burkholderia*', but retrospective analysis reveals that these bacteria were actually *Paraburkholderia* and, to a lesser extent, *Caballeronia* (Table S5) [57, 81–90]. The problem of taxonomic misidentification is compounded by the poor phylogenetic resolution of 16S rRNA gene-based classifications for

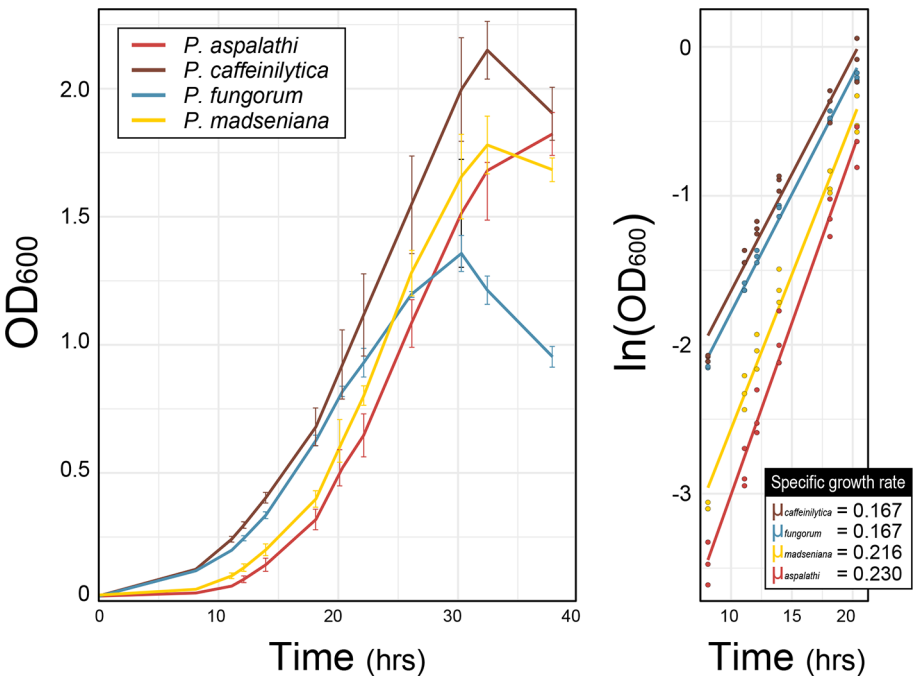

Fig. 2. Growth curves of strain RP11^T and its closest related species of *Paraburkholderia* on MSM media with 4-hydroxybenzoic acid (10 mM) as the sole carbon source. All culturing conditions were selected for the optimum growth of strain RP11^T (pH 7.0 and 30 °C and shaking at 180 r.p.m.).

Paraburkholderia. These taxonomic challenges call for the use of advanced genomic, phylogenetic and chemotaxonomic approaches when describing members of *Paraburkholderia* and the other five genera formerly belonging to *Burkholderia*.

DESCRIPTION OF *PARABURKHOLDERIA MADSENIANA* SP. NOV.

Paraburkholderia madseniana [mad.se.ni.a′na. N.L. fem. adj. *madseniana*, named in honor of the late Dr. Eugene L. Madsen, professor of microbiology at Cornell University, beloved colleague, mentor, and friend, who isolated strain RP11^T in the year before his untimely passing].

Cells are Gram-negative, motile, non-sporulating, rods (0.8 µm by 1.4 µm) that grow primarily as diplobacillus or in short chains (3–4 cells). Colonies are circular and convex with a smooth edge, appearing opaque, lustrous and cream/off-white in colour. Optimum growth occurred at 30 °C and pH 6.5–7.0 on MSM and 10 mM 4-hydroxybenzoic acid with a specific growth rate of 0.216 hr^{-1}. The organism is phylogenetically related to the genus *Paraburkholderia*. Positive reactions are observed for acid phosphatase, ~~alkaline phosphatase~~, esterase (C4), esterase lipase (C8), leucine arylamidase and napathol-AS-BI-phosphohydrolase. Negative reactions for, *N*-acetyl-β-glucosaminidase, α-fucosidase, α-mannosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), and cystine, trypsin and valine arylamidase. Tests were positive for utilization of acetic acid, α-D-glucose, α-hydroxy-butyric acid, α-keto-butyric acid, β-hydroxy-D, L-butyric acid, bromo-succinic acid, citric acid, D-arabitol, D-aspartic acid, D-fructose, D-galactose, D-gluconic acid, D-glucuronic acid, D-malic acid, D-mannitol, D-mannose, raffinose, D-saccharic acid, D-sorbitol, formic acid, γ-amino-butyric acid, glycerol, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-fucose, L-galacturonic acid lactone, L-glutamic acid, L-histidine, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-rhamnose, L-serine, methyl pyruvate, mucic acid, N-acetyl-D-glucosamine, pectin, 4-hydroxy-phenylacetic acid, quinic acid, sucrose, Tween-40, benzoic acid, 4-hydroxybenzoate, 4-coumaric acid, and phthalic acid. Tests were negative for utilization of 3-methyl glucose, acetoacetic acid, lactose, α-keto-glutaric acid, β-methyl-D-glucoside, cellobiose, dextrin, D-fructose-6-phosphate, D-fucose, D-galacturonic acid, D-glucose-6-phosphate, D-lactic acid methyl ester, maltose, melibiose, D-salicin, trehalose, turanose, gelatin, gentiobiose, glucuronamide, inosine, myo-inositol, N-acetyl neuraminic acid, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, propionic acid, stachyose, vanillin, ferulic acid, guaiacol, salicylic acid and syringic acid. The most abundant cellular fatty acids (ordered by abundance) are $C_{17:0}$ cyclo, $C_{16:0,}$ $C_{19:0}$ cyclo ω8c, summed feature 2, $C_{16:0}$ 3-OH, $C_{12:0}$, summed feature 8, $C_{14:0}$, summed feature 3, $C_{16:1}$ 2-OH, $C_{16:0}$ 2-OH and $C_{18:1}$ 2-OH. The sole respiratory quinone is ubiquinone Q-8.

The type strain, RP11^T (=DSM 110123^T=LMG 31517^T) was isolated from the A horizon of an acidic (pH 3.8–4.2) inceptisol in a uniformly planted red pine forest (Dryden, NY. 42.450945,–76.420638). The DNA G+C content of the type strain is 61.3 mol%. The unassembled and assembled genome sequencing data (VOSW00000000) and 16S rRNA gene (MN239497) were assigned to the NCBI BioProject: PRJNA558488.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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