INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Herpell *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004969
DOI 10.1099/ijsem.0.004969

MICROBIOLOGY SOCIETY
OPEN ACCESS

Paraburkholderia dioscoreae sp. nov., a novel plant associated growth promotor

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Abstract

A novel bacterium, designated strain Msb3^T, was recently isolated from leaves of the yam family plant *Dioscorea bulbifera* (*Dioscoreaceae*). Phylogenetic analysis based on the 16S rRNA gene sequence indicated that this strain belonged to the genus *Paraburkholderia* with *Paraburkholderia xenovorans* as nearest validly named neighbour taxon (99.3% sequence similarity towards the *P. xenovorans* type strain). Earlier genome sequence analysis revealed a genome of 8.35 Mb in size with a G+C content of 62.5 mol%, which was distributed over two chromosomes and three plasmids. Here, we confirm that strain Msb3^T represents a novel *Paraburkholderia* species. *In silico* DNA–DNA hybridization and average nucleotide identity (OrthoANIu) analyses towards *P. xenovorans* LB400^T yielded 58.4% dDDH and 94.5% orthoANIu. Phenotypic and metabolic characterization revealed growth at 15 °C on tryptic soy agar, growth in the presence of 1% NaCl and the lack of assimilation of phenylacetic acid as distinctive features. Together, these data demonstrate that strain Msb3^T represents a novel species of the genus *Paraburkholderia,* for which we propose the name *Paraburkholderia dioscoreae* sp. nov. The type strain is Msb3^T (=LMG 31881^T, DSM 111632^T, CECT 30342^T).

INTRODUCTION

The genus *Paraburkholderia* contains species formally classified as *Burkholderia* that were separated from the latter on the basis of phylogenomic evidence [1]. The majority of *Paraburkholderia* species have been isolated from soil and plant roots or nodules but some have been found in aquatic environments and as opportunistic human pathogens, too [2–7]. Bacteria within the genus usually carry large genomes (7–10 Mb) and are metabolically versatile. Notable is the ability of some to nodulate legumes and fix atmospheric nitrogen [8]. Their tendency to occur in acidic soils [9–14] and the capacity to degrade tannins and phenolics as well as polyaromatic hydrocarbons and halogenated phenols [11, 12, 15–19] highlights their role in the decomposition of plant-derived aromatics and their putative role for bioremediation of polluted soils. Many isolates from the rhizosphere display plant growth promoting properties indicating their possible role as biofertilizers [20–24].

The present study was performed to formally classify strain Msb3^T, which was isolated from the phyllosphere of the 'air potato yam', *Dioscorea bulbifera* [6]. Herpell *et al.* [6] demonstrated that this strain is closely related to *Paraburkholderia xenovorans* and reported several genomic, taxonomic, functional and ecological characteristics of this organism. Its genome encodes an impressive combination of features mediating a beneficial plant-associated lifestyle that include biological nitrogen fixation, plant hormone regulation, detoxification of xenobiotics, degradation of aromatic compounds and multiple protein secretion systems [6]. When applied to tomato, strain Msb3^T exhibited significant growth promotion by increasing the total dry biomass by up to 40% [6]. Below, we reiterate basic taxonomic information from Herpell *et al.* [6] and provide comparative phylogenomic, chemotaxonomic and phenotypic analyses to formally classify this strain as a novel *Paraburkholderia* species.

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Keywords: *Burkholderiaceae*; *Dioscorea*; *Paraburkholderia*; plant growth promotion; tomato; yam; plant microbe interaction.
Abbreviations: ANI, average nucleotide identity; CDS, coding sequence; COG, cluster of orthologous groups of proteins; dDDH, digital DNA–DNA hybridization; GI, genomic island; MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; NAB, buffered nutrient agar; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; TSA, trypticase soy agar; TYGS, Type (Strain) Genome Server.
The 16S rRNA gene sequence of strain Msb3^T is publicly available through the GenBank/EMBL/DDBJ accession number LR861108. The whole genome sequence is available under accession numbers LR699553-LR699557.
A supplementary table is available with the online version of this article.
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ISOLATION AND ECOLOGY

Strain Msb3^T was isolated from fresh *Dioscorea bulbifera* (yam family, *Dioscoreaceae*) leaves that were rinsed with water and surface-sterilized [6]. Leaf acumens and sections were ground in a 0.4% sodium chloride solution and serial dilutions were plated on tryptic soy agar medium (TSA) and incubated aerobically at room temperature (ca. 22 °C) in the dark for 48 h. Nearly pure cultures of a single strain were obtained from every leaf acumen extract in several replicate experiments over several years, but not from leaf extracts. Single bacterial colonies were transferred and purified onto TSA agar plates. Bacteria were stored in 30% glycerol in tryptic soy broth at −80 °C after initial identification. Additional details about the isolation are provided in a study describing the plant growth-promoting activity of strain Msb3^T [6]. The culture was identified as a *Paraburkholderia* strain by comparative 16S rRNA and *gyrB* gene sequence analyses. It was designated Msb3^T [6]. The 'main' 16S rRNA gene sequence extracted from the Msb3^T genome (see Genome Features) exhibited 99.3% sequence similarity with that of *P. xenovorans* LB400^T and less than 98.8% towards the type strains of other validly named *Paraburkholderia* species. The 16S rRNA gene-based amplicon sequencing earlier revealed that this organism was a predominant member of the *D. bulbifera* phyllosphere, making up to 25% of the microbial community on leaf acumens [6]. The association was stable over time and reoccurred after completion of the plants' growth cycles, indicating a long-term symbiotic relationship.

GENOME FEATURES

The whole-genome sequence of strain Msb3^T was determined previously using a PacBio Sequel instrument (*Pacific Biosciences of California, Inc., Menlo Park, CA, United States*) [6]. The Msb3^T genome has a cumulative size of 8.35 Mbp and harbours 8199 coding sequences that are distributed over two chromosomes and three plasmids. The G+C content is 62.5 mol%. An in-depth characterization of functional gene content and phylogenetic analyses have been provided in reference [6]. Additional evidence for the delineation of a new species [25] is presented below.

It should be noted that six *rrn* operons were present in the genome sequence of strain Msb3^T. They could be divided into two groups separated by a single SNP at position 1008 (T → C). The three identical copies on chromosome 1 were used to calculate the sequence similarity values presented above. This 'multiple copy number issue' was also found in other *Paraburkholderia* genomes [12] and should be recognized more widely. It adds to a line of evidence that the 16S rRNA gene has rather low discriminatory power and limited applicability as a phylogenetic marker for *Paraburkholderia* [12, 26].

PHYLOGENOMIC ANALYSES

As shown previously [6], *Burkholderia* sp. Ch1-1, a polycyclic aromatic hydrocarbon (PAH) degrading soil isolate [27], is the closest relative of Msb3^T. The genomes of strains Msb3^T and Ch1-1 were uploaded to the Type (Strain) Genome Server (TYGS) [28] for a whole genome-based taxonomic analysis consisting of the determination of closely related type strains and the calculation of the pairwise digital DNA–DNA hybridization (dDDH) values and their confidence intervals using the recommended settings of the Genome-to-Genome Distance Calculator 2.1 [29]. The resulting dataset from TYGS, comprising the genomes of Msb3^T, Ch1-1 and 15 closely related type strains (Table S1, available in the online version of this article), was also used to calculate the average nucleotide identity (ANI) values using the OrthoANIu algorithm [30]. Finally, whole-genome phylogeny was assessed based on 107 single-copy core genes found in a majority of bacteria [31] using bcgTree [32]. Visualization and annotation of the phylogenetic tree was performed using iTOL [33].

The phylogenomic tree was well resolved and showed high bootstrap support on most branches (Fig. 1). In congruence with earlier results [6], the present phylogenomic analysis showed that strain Ch1-1, *P. xenovorans* LB400^T and *Paraburkholderia aromaticivorans* BN5^T were the closest phylogenetic neighbour strains of strain Msb3^T. Both orthoANIu and dDDH values confirmed that *Burkholderia* sp. Ch1-1 represents the same species as strain Msb3^T as it shared dDDH and orthoANIu values of 96.5 and 99.5%, respectively (Table 1). *P. xenovorans* LB400^T shared dDDH and orthoANIu values of 58.4 and 94.5%, respectively. All other *Paraburkholderia* species yielded dDDH and orthoANIu values well below the species delineation thresholds of 70% [29] and 95–96% [30], respectively (Table 1). Altogether, these genomic analyses confirmed that strain Msb3^T represents a novel *Paraburkholderia* species with *P. xenovorans* as nearest neighbour taxon.

COMPARATIVE GENOMIC ANALYSIS BETWEEN STRAINS MSB3^T AND CH1-1

As the closest relative of strain Msb3^T and, according to our *in-silico* analysis, the same species, we sought to include strain Ch1-1 in our polyphasic comparative genomics analysis. In light of the different source of isolation of strain Ch1-1, namely from PAH contaminated soil from sites of former coal gasification plants in Wisconsin [27], it may exhibit very interesting adaptations and high potential for bioremediation. The strain is, however, not available at any commercial strain collection, nor were we able to obtain the isolate through channels of private communication. We therefore decided to include a thorough comparison of the strain's genome with that of strain Msb3^T outlining similarities and differences between the two strains.

Making use of ANVIO's pangenomics suite [34] all protein coding sequences of both genomes were clustered into gene clusters via NCBI's blastp algorithm and using 10 as the MCL inflation parameter. For Msb3^T and Ch1-1, 7465 and 7928 CDSs, respectively, could be used to compute the pangenome of both strains (Fig. 2). Both strains shared 6383 gene clusters comprised of 6549 and 6794 CDSs, respectively. A total of

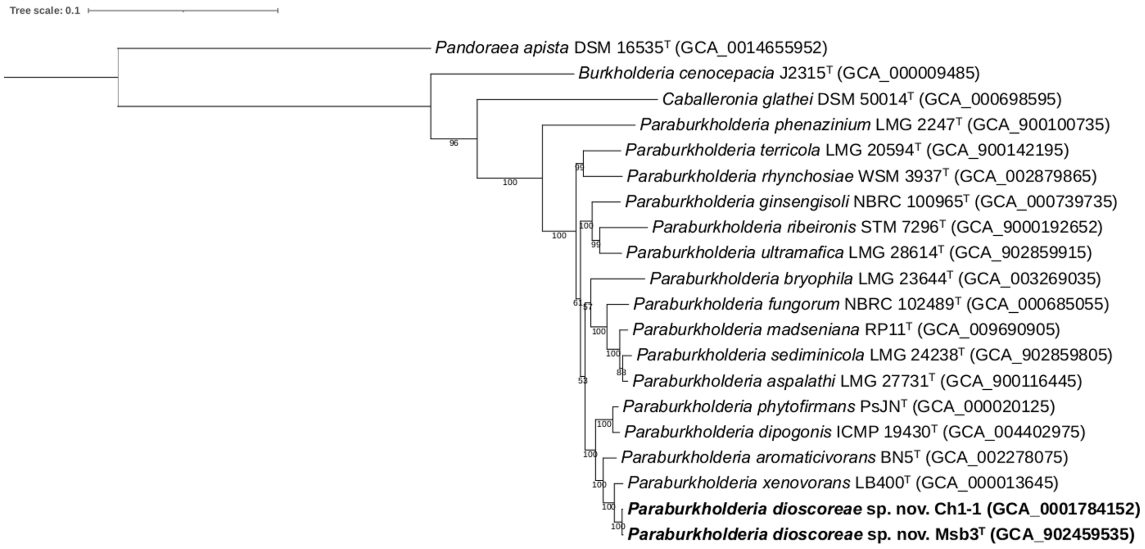

Fig. 1. Phylogenetic tree based on 107 single-copy core genes. BcgTree was used to extract the nucleotide sequence of 107 single-copy core genes and to construct their phylogeny by partitioned maximum-likelihood analysis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. *Pandoraea apista* DSM 16535^T was used as outgroup. Bar, 0.1 changes per nucleotide position.

890 CDSs in 919 clusters from the Msb3^T genome and 1258 CDSs in 1131 clusters from the Ch1-1 genome comprised the accessory genomes of the two strains, accounting for 12.3 and 15.9% of all CDSs, respectively. We analysed the accessory genomes in respect to their functionality. All accessory genes were classified into clusters of orthologous groups of proteins (COGs) [35] and relative differences in COG classification profiles were used to reflect on strain specific biases (Fig. 2). A large part of both accessory genomes consists of CDSs that are either not classifiable or have unknown functions (COG categories R and S), making up 52.8 and 48.4% of the accessory genomes of strain Msb3^T and Ch1-1, respectively. When considering only classifiable sequences, the enrichment of COG categories L (replication, recombination and repair) and D (cell cycle control, cell division, chromosome partitioning) in the accessory genomes of both strains is noteworthy. It could be explained through the presence of unique plasmids. At least in strain Msb3^T, whose genome is closed, all three plasmids display a complete lack of synteny conservation to the genome of strain Ch1-1.

Table 1. Pairwise dDDH and orthoANIu values between strain Msb3^T and its nearest phylogenetic neighbours

Taxon	dDDH (%)	ANI (%)
Paraburkholderia dioscoreae sp. nov. Ch1-1	96.5	99.5
Paraburkholderia xenovorans LB400^T	58.4	94.5
Paraburkholderia aromaticivorans BN5^T	41.8	90.6
Paraburkholderia phytofirmans PsJN^T	37.0	88.8
Paraburkholderia dipogonis ICMP 19430^T	37.1	88.6

The accessory genome of strain Ch1-1 is enriched in category U (intracellular trafficking, secretion, and vesicular transport) with a large number of components of the type IV secretory pathway present. And while the presence of mobile elements is notably high in both accessory genomes, the number of genes that belong to COG category X (mobilome: prophages, transposons) is exceptionally high in strain Ch1-1 making up more than 13% of classifiable CDSs, compared to 7.87% in strain Msb3^T.

The accessory genome of strain Msb3^T, on the other hand, is enriched in CDSs that belong to categories I (lipid transport and metabolism) and M (cell wall/membrane/envelope biogenesis) as well as Q (secondary metabolites biosynthesis, transport and catabolism). Interestingly, many CDSs classified within the latter are located on a genomic island (GI) on chromosome II. The GI is approximately 200 Kbp in length and 83% of its CDSs are not in synteny with the genome of strain Ch1-1. Some of these CDSs share high similarity with the ferredoxin rhodocoxin and the associated gene cluster needed for the degradation of thiocarbamate herbicides via cytochrome P450 [36], a trait which may be useful to explore in a biotechnological context considering the potential of microbial degradation of agrochemicals in the root zone by plant associated bacteria.

Similarly, the *phn* gene cluster in strain Ch1-1, responsible for its capacity to degrade phenanthrene [27, 37], is located on a GI in scaffold 5 of the draft genome assembly and represents another interesting strain specific adaptation.

We also annotated the accessory genomes of both strains via the KEGG database [38] to spot pathways not indicated through a bias in COG category enrichment. Interestingly,

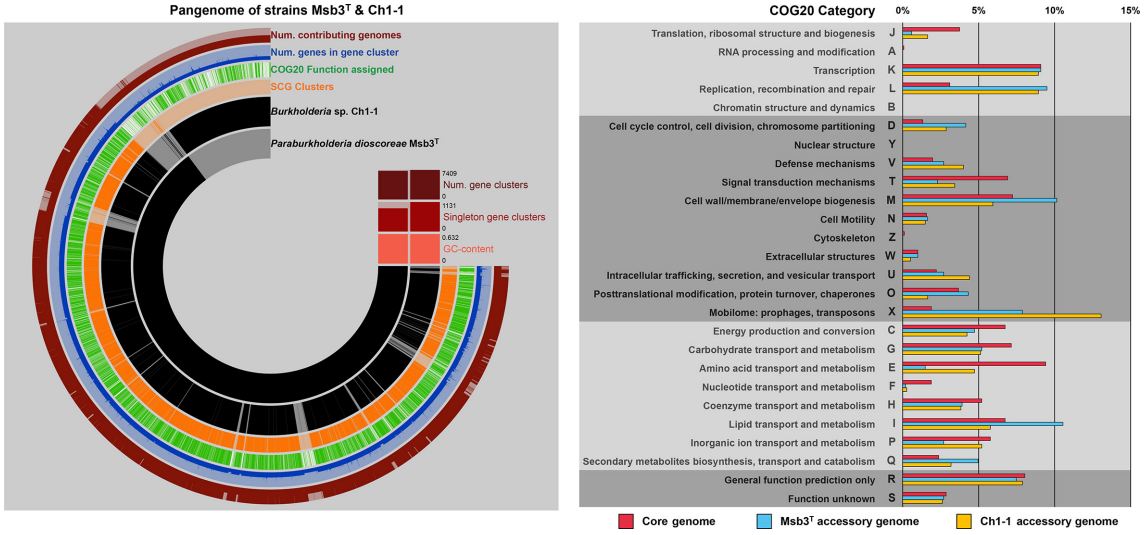

Fig. 2. Pangenome analysis of strains Msb3^T and Ch1-1. The left panel displays the pangenome of both strains created through ANVIO's pangenomics suite [34]. CDSs from strain Msb3^T and strain Ch1-1, respectively, are shown on ring 1 and 2 (starting inside). Presence of a CDS in either genome is indicated in black and absence in light grey. CDSs are forced into synteny with the genome sequence of strain Msb3^T. Ring 3 displays whether two orthologs fall into self-consistency-grouping (SCG) clusters, ring 4, whether a COG20 function could be assigned to the CDS in the cluster. Ring 5 displays the number of genes that fall into a respective gene cluster, ranging from a minimum of one to a maximum of five, in form of a bar chart. The outermost ring, ring 6, indicates, how many genomes contribute to a gene cluster. If two genomes (full bar) contribute to a cluster, it was considered part of the core genome of the two strains. If, however, only one genome contributes (half bar), the respective CDS is considered part of that genome's accessory genome (AG). The panel on the right shows the relative COG category classification patterns of the core genome of both strains in pink, the AG of strain Msb3^T in light blue as well as the AG of strain Ch1-1 in yellow. The functional annotation into COG categories was conducted through the software package ANVIO, which implements the latest update of the COG database, COG20 [35]. Depicted is the percentage of all classifiable CDSs that fall into a given COG category.

strain Ch1-1 carries orthologs of *cciI* and *cciR*, responsible for N-acylhomoserine lactone (AHL) synthesis and its response regulator gene. These genes are usually found in species of the *Burkholderia cepacia* complex (Bcc) on a GI designated cenocepacia island [39] and affect their virulence and capacity to form biofilms [40, 41]. Both strains carry several unique genes connected to plant-microbe-interactions: while a gene encoding the flg22 epitope (*fliC*) is present in strain Msb3^T but not in strain Ch1-1, an ortholog to the rice Xa21-immune response elicitor raxA is present in strain Ch1-1. However, most adaptations to a plant associated lifestyle are shared between both strains.

The two strains also display several interesting adaptations towards degradation of various phenolics. While both strains have the ability to degrade benzoate via catechol to acetyl-coA, only strain Ch1-1 has the capacity to completely degrade hydroxyquinol, 1,2,4-benzenetriol, nitrobenzene and 2-bromo-maleylacetate. Strian Ch1-1 also carries genes from the *bph* gene cluster to catalyse the degradation of polychlorinated biphenyl (PCB) and biphenyl itself. Both strains, however, lack the gene *bphD*, the 2,6-dioxo-6-phenylhexa-3-enoate hydrolase, necessary to complete PCB degradation to benzoate. Strain Ch1-1 certainly has a larger variety of genes encoding enzymes that take part in degradation of PAHs and other phenolic compounds, although many pathways are not complete.

Overall, the two genomes share a very high degree of similarity, indicative of a very close phylogenetic relationship. While the accessory genomes of both strains seem to be shaped by their respective habitat, leading to differences in metabolic potential, the general array of functions predicted on the basis of their genome seem to overlap (Fig. 2). We therefore propose that strain Ch1-1 is a different strain of the same species as strain Msb3^T, as supported by dDDH and orthoANIu values (Table 1). We refrain, however, from describing it to the same degree of detail as strain Msb3^T, seeing as we could not get a hold of a culture to include it in chemotaxonomic and physiological analyses.

PHYSIOLOGY AND CHEMOTAXONOMY

A physiological characterization of strain Msb3^T was performed earlier [6] and is presented below as part of the species description. An additional phenotypic characterization of strain Msb3^T and of the type strains of its two nearest neighbour taxa, i.e. *P. xenovorans* LMG 21463^T and *P. aromaticivorans* LMG 31771^T was performed in the present study. All growth tests were recorded after 2, 3, 5 and 7 days of incubation; but test results after 2 days of incubation are presented below unless specified otherwise.

Cell and colony morphology were assessed after 3 days of incubation on phosphate buffered (0.45 g l^{-1} KH_2PO_4/2.39 g

Table 2. Differential phenotypic characteristics of strain LMG 31881^T and closely related type strains. All data are from the present study. +, Positive reaction; W, weakly positive reaction; –, negative reaction; (+), delayed positive

Characteristic	*P. dioscoreae*	*P. xenovorans*	*P. aromaticivorans*
	LMG 31881^T (Msb3^T)	**LMG 21463^T**	**LMG 31771^T**
Growth at:			
15 °C on TSA	+	–	+
37 °C on NAB	w	+	w
Growth in the presence of 1% NaCl	+	–	+
Activity of:			
Catalase	+	+	(+)
Oxidase	+	+	w
Activity of (API 20NE): beta-Galactosidase	–	–	+
Assimilation of (API 20NE):			
L-Arabinose	w	–	+
Capric acid	+	+	–
Phenylacetic acid	–	+	+

l^{-1} Na_2HPO_4.12H2O) nutrient agar [42] (Oxoid) at 28 °C. Motility was observed in young cultures by examining wet mounts in broth by phase-contrast microscopy. The assessment of catalase and oxidase activities and other tests were performed using conventional procedures [43]. Aerobic growth was tested at 28 °C on NAB, TSA, MacConkey agar (Oxoid), Drigalski agar (Biorad) and *Pseudomonas* cetrimide agar (Oxoid). The temperature growth range was tested on NAB and TSA at 4, 15, 20, 28, 37, 40 and 45 °C. The effect of NaCl on growth was investigated in nutrient broth (Oxoid) with different concentrations of NaCl (0–10% with 1% intervals, w/v). The pH range for growth was evaluated in nutrient broth buffered at pH 4.0 to 9.0 at intervals of 1 pH unit using the following buffer systems: acetate buffer (4.0–5.0), phosphate buffer (pH 6.0–8.0) and Tris-HCl (pH 9.0). Anaerobic growth was tested on NAB, TSA and TSA supplemented with 10 mM KNO_3. Hemolysis of horse blood was tested using TSA supplemented with 5% horse blood. DNase activity was tested using DNase agar (Sigma-Aldrich). Starch hydrolysis was tested using NAB and TSA supplemented with 0.8% (w/v) soluble starch. Hydrolysis of casein was tested using Plate count agar (Oxoid) supplemented with 1.3% (w/v) dried skim milk (Oxoid). Hydrolysis of Tween 20, 40, 60 and 80 was tested as described by Sierra (1957). The activity of constitutive enzymes and other physiological properties were determined after growth on TSA for 2 days at 28 °C using the API 20NE and API ZYM microtest systems (BioMérieux), according to the manufacturer's instructions. Results were read after 24 h and 48 h, and after 4 h of incubation, respectively.

The three strains shared the following characteristics: growth on MacConkey and Drigalski agar and on Tween 20, 40, 60 and 80 agar base. Growth in the presence of 0% NaCl and at pH 6 and 7. Growth at 20 and 28 °C on TSA and at 15, 20 and 28 °C on NAB. Hemolysis on horse blood agar after 5 days of incubation. Activities of the following enzymes were present: aesculin hydrolysis, assimilation of D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, adipic acid, malate and trisodium citrate (API 20NE), and butyrate esterase (C4) (weak), caprylate esterase lipase (C8) (weak), alkaline and acid phosphatase, phosphoamidase and leucyl arylamidase (API ZYM).

The following characteristics were uniformly absent: growth on *Pseudomonas* cetrimide agar, hemolysis on horse blood agar after 2 and 3 days of incubation. DNase activity, hydrolysis of starch, casein and Tween 20, 40, 60 and 80. Anaerobic growth on NAB, TSA or TSA supplemented with 10 mM KNO_3. Growth in the presence of 2–10% NaCl and at pH 4.0, 5.0, 8.0 and 9.0. Growth at 4, 37, 40 and 45 °C on TSA and growth at 4, 40 and 45 °C on NAB. When examined using the API 20NE microtest system, the following characteristics were absent: nitrate reduction, indol production from L-tryptophane, fermentation of glucose, activity of arginine dihydrolase and urease, gelatin liquefaction and assimilation of maltose. When examined using the API ZYM microtest system, the following enzyme activities were absent: myristate lipase (C14), valine arylamidase, cystin arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase and alpha-fucosidase.

Table 2 presents biochemical characteristics that allow to differentiate strain Msb3^T from *P. xenovorans* LMG 21463^T and *P. aromaticivorans* LMG 31771^T. Growth at 15 °C on TSA, growth in the presence of 1% NaCl and the lack of assimilation of phenylacetic acid allowed to distinguish strain Msb3^T

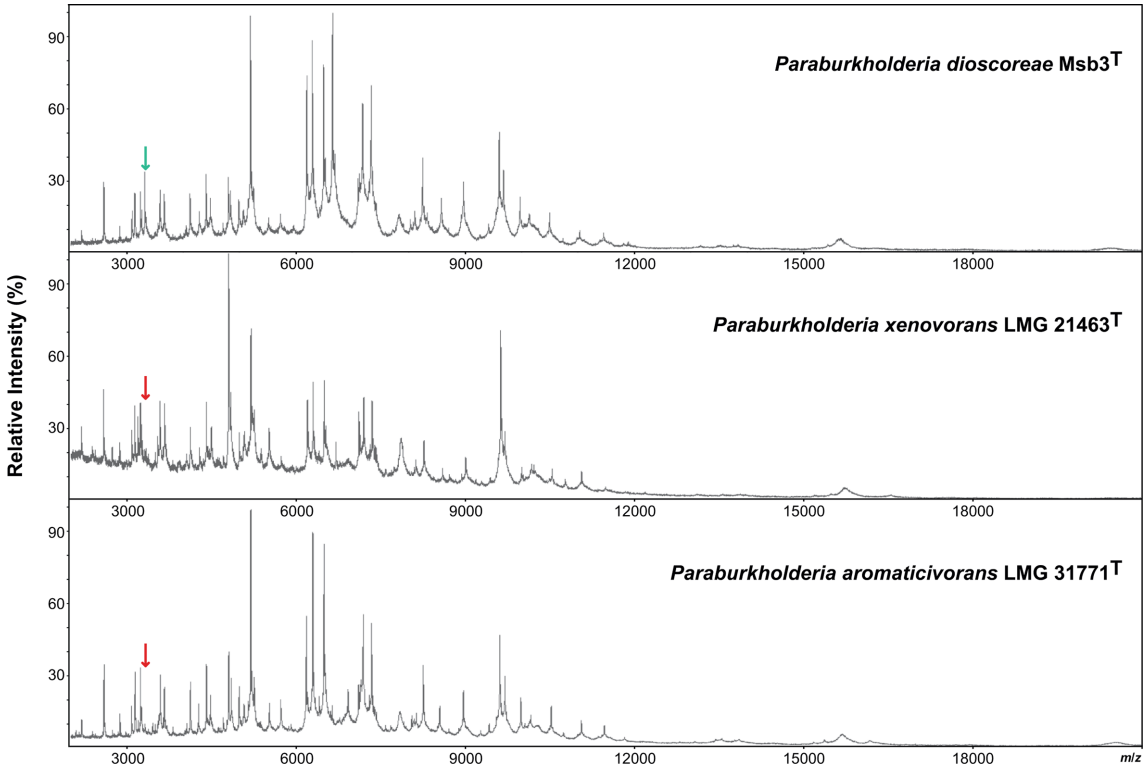

Fig. 3. MALDI-TOF MS profiles of strains Msb3^T, *P. xenovorans* LMG 21463^T and *P. aromaticivorans* LMG 31771^T. Peaks were visualized by mMass v. 5.5.0 [49]. The peak at *m/z* 3321.8 allows to distinguish strain Msb3^T from *P. xenovorans* LMG 21463^T, as indicated by the green (present) and red (absent) arrows.

from the former; catalase and oxidase activity, the assimilation of capric acid and the lack of assimilation of phenylacetic acid allowed to distinguish strain Msb3^T from the latter.

Finally, MALDI-TOF MS profiles of strains Msb3^T from *P. xenovorans* LMG 21463^T and *P. aromaticivorans* LMG 31771^T were obtained as described before [44]. All strains were cultivated on NAB at 28 °C for 2 days before cell extracts were prepared. Strains Msb3^T and *P. xenovorans* LMG 21463^T had highly similar MALDI-TOF MS profiles but could be differentiated by a peak at m/z 3321.8 in the former profile. *P. aromaticivorans* LMG 31771^T had a clearly distinct MALDI-TOF MS profile (Fig. 3). Today, MALDI-TOF MS is used worldwide as the leading diagnostic instrument for species level identification of medical, pharmaceutical and food-related bacteria [45, 46] and its use for studying environmental ecosystems is increasing rapidly [47]. The data produced in the present study demonstrated that MALDI-TOF MS reference libraries, supplemented with reference spectra of the *Paraburkholderia* species studied, will facilitate species level identification. Unlike other chemotaxonomic markers routinely included in polyphasic taxonomic studies today [48] this provides practical diagnostic value.

In conclusion, the present phylogenomic (Figs 1 and 2, Table 1) and phenotypic (Table 2, Fig. 3) analyses confirmed that strains Msb3^T and Ch1-1 represent a novel *Paraburkholderia* species that can be distinguished genotypically from the type strains of *P. xenovorans* and *P. aromaticivorans,* its nearest phylogenetic neighbour taxa. Additionally, chemotaxonomic and phenotypic analyses of the type strain, Msb3^T, could confirm this distinction. We therefore propose to formally classify this strain into the novel species *Paraburkholderia dioscoreae* sp. nov., with Msb3^T (=LMG 31881^T, DSM 111632^T, CECT 30342^T) as the type strain.

DESCRIPTION OF *PARABURKHOLDERIA DIOSCOREAE* SP. NOV.

Paraburkholderia dioscoreae sp. nov. (Di.os.co.re'ae. N.L. gen. n. *Dioscoreae*, of *Dioscorea*, referring to the plant genus source of isolation).

Cells are Gram-stain-negative, motile and rod-shaped. Optimal growth of strain Msb3^T is observed between 22 and 28 °C in aerobic conditions. After 3 days of incubation on NAB at 28 °C, colonies are about 1 mm in diameter, beige, opaque, flat, circular with a smooth surface and an entire edge; cells are plump rods that are 1 to 2 µm long and about 0.5 µm wide. No anaerobic growth on NAB, TSA or TSA supplemented with 10 mM KNO_3. Growth on rich medium is inhibited between 36 and 37 °C. A clear growth rate reduction was observed at temperatures between 30 and 35 °C. Growth at 15, 20 and 28 °C on TSA, but not at 4, 37, 40 or 45 °C after 7 days of incubation; growth at 15, 20, 28 and 37 °C (weak)

on NAB, but not 4, 40 or 45 °C after 7 days of incubation. Growth in the presence of 0 and 1% NaCl, but not 2% or more. Growth at pH 6 and 7, but not at pH 4.0, 5.0, 8.0 or 9.0.

The preferred carbon sources of strain Msb3^T are simple C4 sugars. The strain can grow in minimal medium that contains glucose as well as malate and citrate as sole carbon sources. At an equimolar concentration growth on malate is more efficient than growth on glucose. Growth on MacConkey and Drigalski agar, but not on *Pseudomonas* cetrimide agar. Growth on Tween 20, 40, 60 and 80 agar base; hydrolysis of Tween 20, 40, 60 and 80 was apparent after 3 days of incubation only. No DNase activity or hydrolysis of starch or casein. Hemolysis on horse blood agar was negative after 2 days of incubation but positive after 5 days of incubation. Catalase and oxidase activity are present.

When examined using the API 20NE microtest system, the following characteristics were present in strain Msb3^T: aesculin hydrolysis, assimilation of L-arabinose (weakly), D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, adipic acid, malate, caprate and trisodium citrate but not nitrate reduction, indol production from L-tryptophane, beta-galactosidase (ONPG), fermentation of glucose, activity of arginine dihydrolase, urease and gelatin liquefaction, and assimilation of maltose or phenylacetic acid.

When examined using the API ZYM microtest system, the following characteristics were present in strain Msb3^T: butyrate esterase (C4) (weak), caprylate esterase lipase (C8) (weak), alkaline and acid phosphatase, phosphoamidase and leucyl arylamidase, but not myristate lipase (C14), valine arylamidase, cystin arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase or alpha-fucosidase.

Strain Msb3^T is resistant to several commonly used antibiotics including ampicillin (50–100 μg ml^{-1}), cefalexin (10–40 μg ml^{-1}), and chloramphenicol (25 μg ml^{-1}). It is, however, sensitive to standard inhibitory concentrations of carbenicillin, streptomycin as well as kanamycin.

The type strain is Msb3^T (=LMG 31881^T, DSM 111632^T, CECT 30342^T), which was isolated in December 2017 from leaf acumens of *Dioscorea bulbifera*. The whole-genome sequence of strain Msb3^T has a size of 8.35 Mbp and consists of two chromosomes and three plasmids. The G+C content is 62.5 mol%. This whole genome sequence is publicly available under accession numbers LR699553-LR699557 (BioProject PRJEB33427). The 16S rRNA gene sequence of strain Msb3^T is publicly available through the GenBank/EMBL/DDBJ accession number LR861108.

Funding information
The authors received no specific grant from any funding agency.

Acknowledgements
We thank Margo Cnockaert for technical assistance. The Vienna Biocenter Core Facility (VBCF) (University of Vienna) is acknowledged for providing essential services for the generation of the presented sequencing data. We are grateful for the hard work of our gardeners Andreas Schröfl and Thomas Joch.

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

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