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ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Burkholderia perseverans sp. nov., a bacterium isolated from the Restinga ecosystem, is a producer of volatile and diffusible compounds that inhibit plant pathogens

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Abstract

Gram-negative, aerobic, rod-shaped, non-spore-forming, motile bacteria, designated CBAS 719 ^{T}, CBAS 732 and CBAS 720 were isolated from leaf litter samples, collected in Espírito Santo State, Brazil, in 2008. Sequences of the 16S rRNA, *gyrB*, *lepA* and *recA* genes showed that these strains grouped with *Burkholderia plantarii* LMG 9035 ^{T}, *Burkholderia gladioli* LMG 2216 ^{T} and *Burkholderia glumae* LMG 2196 ^{T} in a clade of phytopathogenic *Burkholderia* species. Digital DNA-DNA hybridization experiments and ANI analyses demonstrated that strain CBAS 719 ^{T} represents a novel species in this lineage that is very closely related with *B. plantarii*. The genome sequence of the type strain is 7.57 Mbp and its G + C content is 69.01 mol%. The absence of growth on TSA medium supplemented with 3% (w/v) NaCl, citrate assimilation, β-galactosidase (PNPG) activity, and of lipase C14 activity differentiated strain CBAS 719 ^{T} from *B. plantarii* LMG 9035 ^{T}, its nearest phylogenetic neighbor. Its predominant fatty acid components were $C_{16:0}$, $C_{18:1}$ ω7c, cyclo-$C_{17:0}$ and summed feature 3 ($C_{16:1}$ ω7c and/or $C_{15:0}$ *iso* 2-OH). Based on these genotypic and phenotypic characteristics, the strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 are classified in a novel *Burkholderia* species, for which the name *Burkholderia perseverans* sp. nov. is proposed. The type strain is CBAS 719 ^{T} (= LMG 31557 ^{T} = INN12^{T}).

Keywords *Aspergillus welwitschiae* · *Burkholderia plantarii* · Genome analysis · Sisal

The genus *Burkholderia* was proposed by Yabuuchi et al. [1] as a member of the family *Burkholderiaceae* [2]. This genus is diverse and comprises more than 100 validly named species in the List of Prokaryote names with Standing in Nomenclature (LPSN; www.bacterio.net/burkholderia.html) [3]. Due to its phylogenetic diversity, this genus has recently been subdivided into *Burkholderia* sensu stricto (which

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Accession numbers: The DDBJ/EMBL/GenBank accession numbers of *Burkholderia perseverans* CBAS 719 ^{T} (=LMG 31557 ^{T}=INN12^{T}) for the 16S rRNA gene sequence is KY365423, and the draft genome sequences are CP045094—CP045093.

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comprises species of the *Burkholderia cepacia* complex (BCC), *B. pseudomallei* group species and several phytopathogenic species) and the novel genera *Paraburkholderia*, *Caballeronia*, *Robbsia*, *Mycetohabitans* and *Trinickia* [4–7]. *Burkholderia* species occupy different niches and may be isolated from plants, animals, soils and leaf litter [7–9]. These bacteria can be pathogens to plants and animals; however, many *Burkholderia* species may be beneficial, acting as biological control agents and plant growth promoters [8–10].

Isolation and ecology

In 2008, three leaf litter samples were collected in a sandbank located at Parque Estadual Paulo Cesar Vinha, Espírito Santo State, Brazil (20° 35′ 23″ S 40° 24′ 40″ W) [10]. This region is part of the Restinga ecosystem, a Brazilian coastal biome located in the eastern part of the country. It is characterized by sandy, nutrient-poor and acidic soils with heavy influences from the Atlantic sea and the Atlantic forest. The biodiversity and biotechnological potential of the microbiota from this biome have been systematically studied by our research group [10–14]. Each sample, containing 10 g, was washed 15 times in running water, three times in water with a drop of tween 20 and two times in distilled water. Then, samples were mixed with 90 mL of distilled water, grinded for 1 min in a blender and tenfold serial dilutions were prepared. Aliquots of 100 µL were spread on malt extract agar (MEA; Acumedia) plates containing chloramphenicol (70 ppm). After 2 days of incubation at 28 °C, colonies were randomly selected and subcultured on LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl and 15 g agar). Pure cultures were maintained at − 80 °C in 40% (v/v) glycerol.

16S ribosomal RNA phylogeny

The collection included a group of 16 strains with 100% identical 16S rRNA gene sequences, which were selected for further studies. Genotyping with BOX-PCR showed that all strains were identical [10]. Strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 were selected because they were shown to produce volatile organic compounds (VOCs) with activity in vitro against *Aspergillus welwitschiae* and lowered the severity of bole rot of sisal (*Agave sisalana*) by 75–79% in field experiments [10]. The 16S rRNA gene sequences of strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 were 99.5% identical to those of *Burkholderia plantarii* LMG 9035 ^{T}, and 99.4% identical to both *Burkholderia gladioli* LMG 2216 ^{T} and *Burkholderia glumae* LMG 2196 ^{T} (Fig. 1).

gyrB, *lepA* and *recA* genes phylogeny

Partial sequences of the *gyrB*, *lepA* and *recA* genes of strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 were obtained using the method described by Spilker et al. [15]. The PCR products were sequenced using an AB3500 sequencer according to the manufacturer's instructions (Applied Biosystems). Sequences were assembled and edited with Sequencher v. 5.4.6 (Gene Codes Corporation) and were compared with sequences deposited in public databases using the BLAST

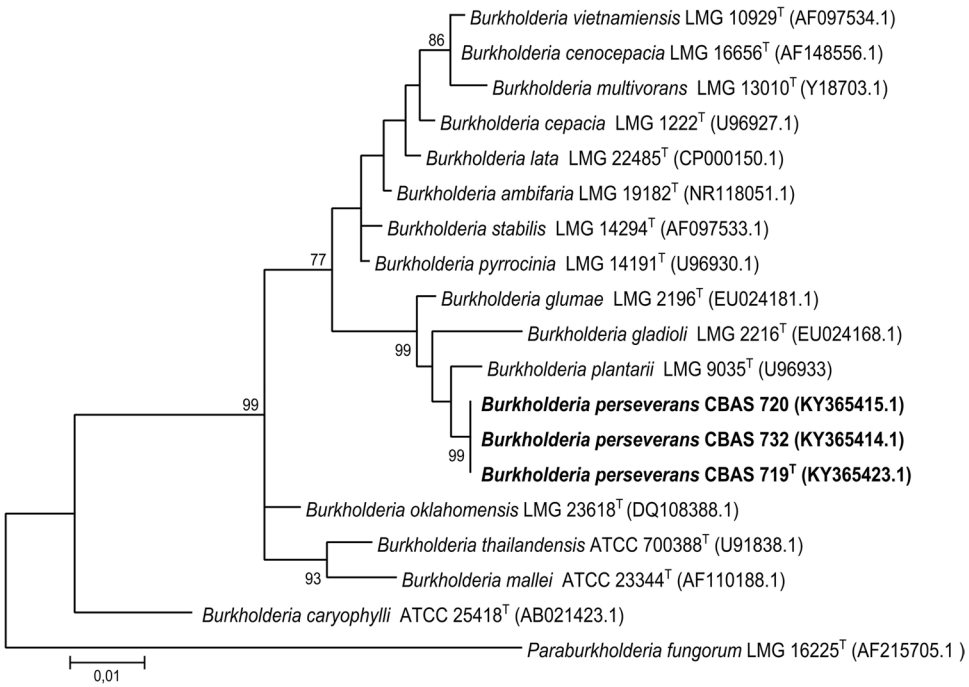

Fig. 1 Maximum likelihood tree based with 1118 ungapped positions of the multiple alignment of the 16S rRNA gene of *Burkholderia perseverans* sp. nov. and related species. The phylogenetic analysis was performed employing the Tamura-Nei substitution model with Gamma distribution and Invariant sites. Bootstrap analysis employed 1000 re-samplings; only bootstrap support values above 70% are presented at the nodes. *Paraburkholderia fungorum* LMG 16225 ^{T} was used as outgroup. The scale bar indicates the number of substitutions per site. The new species is presented in bold font (^{T} = ex-type). GenBank accession numbers are given between parentheses

program [16]. All multiple alignments and phylogenetic analyses with the maximum likelihood method were performed using MEGA v.6.0 software [17]. In an analysis of combined *gyrB*, *lepA* and *recA* gene sequences, strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 were in a clade with a 100% bootstrap support (Fig. 2) containing *B. plantarii* LMG 9035 ^{T}, *B. gladioli* LMG 2216 ^{T} and *B. glumae* LMG 2196 ^{T}, all phytopathogenic species. *Burkholderia gladioli* and *Burkholderia glumae* are basal in this clade indicating that *B. plantarii* LMG 9035 ^{T} and the taxon represented by the strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 evolved more recently (Fig. 2). Similar results were also found in phylogenetic analyses performed for each gene separately (data not shown).

Genome features

The whole genome sequence of strain CBAS 719 ^{T} was obtained in an Illumina Hiseq platform (Macrogen) using the 125-bp paired-end TruSeq DNA PCR-free library kit. Sequencing yielded 30,766,864 reads, of which the quality was verified using the FastQC (v.0.11.5) program [18]. SPAdes (v.3.11.1) software was used for de novo assembly of the reads [19]. Then, the contigs were ordered and extended into scaffolds using the software CONTIGuator (v.2.7.4) [20] and the *B. plantarii* LMG 9035 ^{T} (accession number GCA_001411805.1) genome sequence was used as reference. The gap-closure procedures were conducted using the tool FGAP (v.1.8.1) [21], BLASTn [16] and CLC Genomics Workbench (v.7.0) (Qiagen Inc.) respectively. The draft genome assembly is available in the GenBank database under accession numbers CP045094—CP045093.

The G + C content of strain CBAS 719 ^{T} as calculated from its genome was 69.01 mol%, a value similar to that of its closest neighboring species (Table 1). Digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values were calculated using the Genome-to-Genome Distance Calculator 2.1 provided by the Leibniz Institute DSMZ website (http://ggdc.dsmz.de/distcalc2.php) and the JSpeciesWS web service (http://jspecies.ribohost.com/jspeciesws/#analyse) [22] with the recommended parameters and/or default settings, respectively. The dDDH value between strain CBAS 719 ^{T} and *B. plantarii* LMG 9035 ^{T} was 60.5%

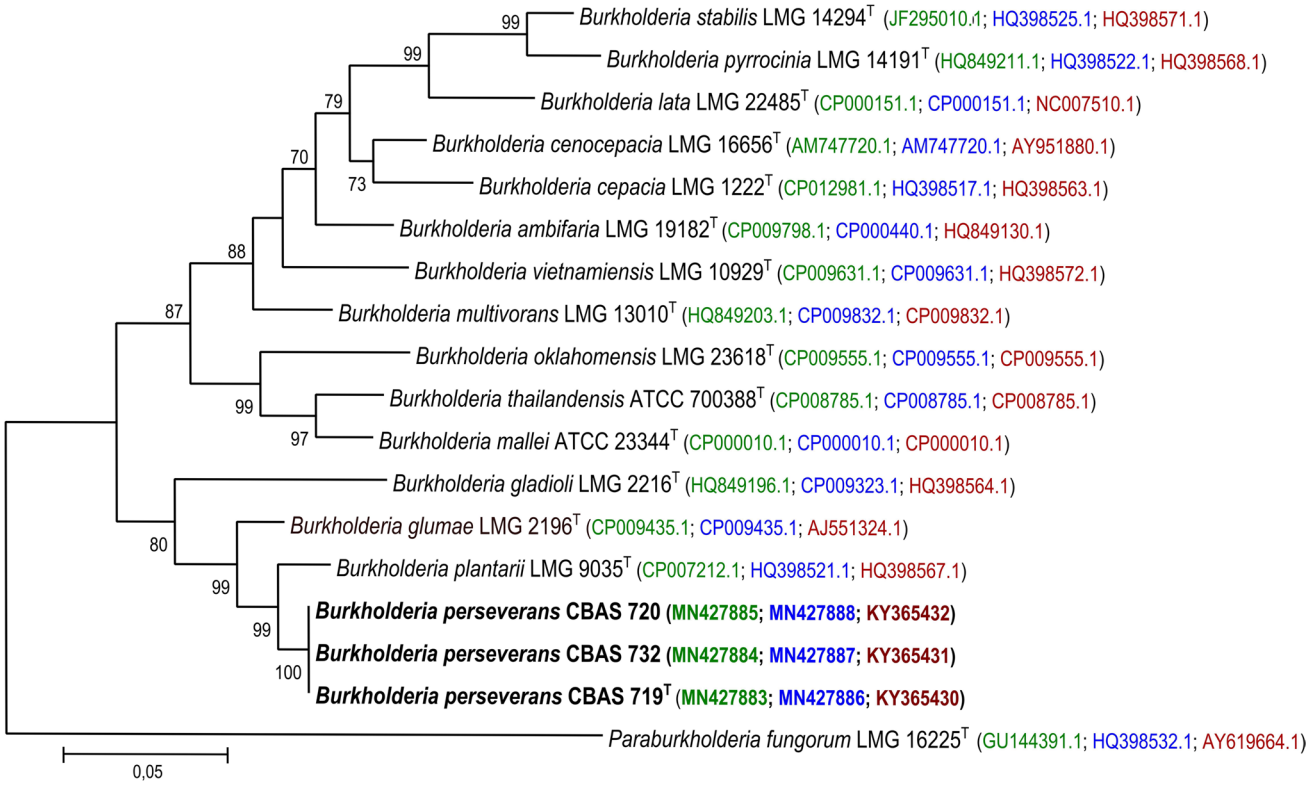

Fig. 2 Maximum likelihood tree based on 1524 ungapped positions of the combined multiple alignment of *gyrB*, *lepA* and *recA* genes from *B. perseverans* sp. nov. and phylogenetically related species of the genus *Burkholderia*. The phylogenetic analysis was performed using the Tamura 3-parameter substitution model with Gamma distribution. Bootstrap analysis employed 1000 re-samplings; only bootstrap support values above 70% are presented at the nodes. *Paraburkholderia fungorum* LMG 16225 ^{T} was used as outgroup. The scale bar indicates the number of substitutions per site. The new species is presented in bold font (^{T} = ex-type). GenBank accession numbers are given between parentheses (*gyrB* = green, *lepA* = blue, *recA* = red)

Table 1 dDDH and ANI values between the genome of *B. perseverans* CBAS 719 ^{T} as query genome and that of closely related species. ANI values were calculated using JSpecies with the ANIb algorithm (average nucleotide identity based on BLAST). Numbers between parentheses after ANI values are percentages of conserved aligned DNA between two genomes; numbers between parentheses after dDDH values are the confidence intervals

Query genome	Reference genome	Bioproject NCBI number	Size (mb)	G + C (mol %)	dDDH (%)	ANI (%)
B. perseverans CBAS 719 ^{T}	*B. perseverans* CBAS 719 ^{T}	PRJNA573627	7.57	69.01	100	100
B. perseverans CBAS 719 ^{T}	*B. plantarii* LMG 9035 ^{T}	PRJNA237833	8.08	68.55	60.5 (57.7–63.3)	94.8 (75.9)
B. perseverans CBAS 719 ^{T}	*B. glumae* LMG 2196 ^{T}	PRJNA259679	6.82	68.18	44.8 (42.2–47.3)	91.2 (53.8)
B. perseverans CBAS 719 ^{T}	*B. gladioli* LMG 2216 ^{T}	PRJNA238809	8.90	67.63	29.6 (27.3–32.1)	85.1 (53.9)

(with 57.7–63.3% as confidence interval); the corresponding ANI value was 94.8%. Both values were near, but below, the thresholds of 70% dDDH [23, 24] and 95% ANI [24] for bacterial species delineation, indicating that strain CBAS 719 ^{T} represents a distinct species (Table 1).

Physiology and chemotaxonomy

Phenotypic analyses of strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 and of the type and reference strains of *B. plantarii*, *B. gladioli* and *B. glumae* (Table 2) were performed after cultivation of cells on tryptone soya agar (TSA, Oxoid) at 28 °C unless indicated otherwise. Cell morphology and motility were observed by phase-contrast microscopy. Oxidase activity was detected by immersion of cells in 1% *N,N,N',N'*-tetramethyl *p*-phenylenediamine solution and catalase activity was determined by bubble formation after flooding colonies with 10% H_2O_2. Lipase activity was determined according to the method described by Sierra [26]. Growth on MacConkey agar was observed after 48 h of incubation at 28 °C. Starch hydrolysis was observed after 48 h of incubation at 28 °C in TSA supplemented with 2% starch. DNase activity was assessed after 48 h of incubation at 28 °C on DNase test agar (BD Difco), according to the method of Jeffries et al. [27]. Casein hydrolysis was determined after 48 h of incubation at 28 °C on TSA plates supplemented with 1.3% skimmed milk, through the observation of clear haloes around colonies. Growth on cetrimide and blood agar medium was observed after 48 h of incubation at 28 °C. Growth on medium with tween 20, 40, 60 and 80 was observed after 24 h of incubation at 28 °C. Nitrate reduction was determined on TSA medium supplemented with 10 mM KNO_3. Growth was tested at 28 °C in nutrient broth (BD Difco) at pH 4–9 using appropriate biological buffers (acetate, citrate/Na_2HPO_4, phosphate buffer and Tris/HCl).

Growth on TSA was tested at 4, 15, 20, 28, 37, 40, 42 and 45 °C (aerobic conditions), and at 28 °C in anaerobic conditions using the Anaero Pack system (Mitsubishi Gas Chemicals). Growth in tryptone soya broth was tested in the presence of 0–10.0% (w/v) NaCl at intervals of 1.0% units. Other biochemical tests were performed by inoculating in API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions and incubating for 48 h at 28 °C or for 4 h at 28 °C, respectively. The results of the biochemical analyses, and in particular lipase C_{14} activity, differentiated the novel taxon from *B. plantarii* LMG 9035 ^{T} (Table 2). In addition, although variable reactions were observed in the novel taxon, the type strain CBAS 719 ^{T} could further be distinguished from the *B. plantarii*–type strain by the absence of growth on TSA medium supplemented with 3% (w/v) NaCl, citrate assimilation and of β-galactosidase (PNPG) activity.

Whole-cell fatty acid methyl esters were extracted according to the MIDI protocol (http://www.youngin.com/application/AN-0505-0002EN.pdf). After a 24-h incubation period at 28 °C on trypticase soya broth (BD Difco) supplemented with 1.5% (w/v) bacto™ agar (BD Difco), a loopful of cells was harvested and fatty acid methyl esters were prepared. The profiles were generated using an Agilent Technologies 6890 N gas chromatograph and identified and clustered using the Microbial Identification System software and MIDI TSBA database v.5.0. The analysis revealed that the most abundant fatty acids in the strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 and in the *B. plantarii*, *B. gladioli* and *B. glumae* reference strains were $C_{16:0}$, $C_{18:1}$ ω7c, cyclo-$C_{17:0}$ and summed feature 3 ($C_{16:1}$ ω7c and/or $C_{15:0}$ *iso* 2-OH) (Table S1). The fatty acid components that represented more than 1% of the total were as follows: $C_{12:0}$, $C_{13:1}$, $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ ω7c, cyclo-$C_{17:0}$, cyclo-$C_{19:0}$ ω8c, $C_{16:0}$ 3-OH, $C_{18:1}$ 2-OH, summed feature 2 ($C_{12:0}$ aldehyde and/or $C_{14:0}$ 3-OH and/or $C_{16:1}$ *iso*) and summed feature 3 ($C_{16:1}$ ω7c and/or $C_{15:0}$ *iso* 2-OH). The most discriminating fatty acids between the novel taxon represented by the strains CBAS 719 ^{T}, CBAS 732 and CBAS 720 and *B. plantarii* were $C_{13:1}$, $C_{18:1}$ ω7c, cyclo-$C_{17:0}$, cyclo-$C_{19:0}$ ω8c and summed feature 3 (Table S1). The overall fatty acid profile of the new taxon supports its placement in the genus *Burkholderia* [1].

Strains were cultured twice on nutrient agar prior to MALDI-TOF MS analysis. Cell pellets and extracts for MALDI-TOF MS were prepared as described by Wieme et al. [25]. Cell extracts (1 µL) were spotted in duplicate on a Bruker target plate. Subsequently, the spots were

Table 2 Differential biochemical characteristics of *B. perseverans* sp. nov. and phylogenetically related species. Species: (1) *B. perseverans* sp. nov. CBAS 719 [T], CBAS 732 and CBAS 720; (2) B. *plantarii* LMG 9035 [T] and LMG 10,911; (3) B. *gladioli* LMG 2216 [T], LMG 11,626 and LMG 18,920; (4) B. *glumae* LMG 2196 [T], LMG 19,583 and R-21928. All data were obtained in the present study. +, positive; *w*, weakly positive; *v* +, variable among strains of the species but the type strain has the ability; *v* −, variable among strains of the species but the type strain does not have the ability; *vw*, variable among strains but weak in type strain; *w* −, weak in some strains but negative in type strain; −, negative. Culture medium: MacConkey Agar, + fermented lactose, − lactose is not fermented, *v* + variable among strains of the species but the type strain fermented lactose, *v* − variable among strains of the species but the type strain lactose is not fermented; blood agar, + causes hemolysis, − does not cause hemolysis, *v* + variable among strains of the species but the type strain causes hemolysis, *v* − variable among strains of the species but the type strain does not cause hemolysis

Characteristic	1	2	3	4
Growth at:				
40 °C	w	vw	+	+
pH 8	−	v −	w	w
3% NaCl	v −	+	v +	+
4% NaCl	−	v −	v +	w
5% NaCl	−	v −	w	vw
6% NaCl	−	−	vw	−
7% NaCl	−	−	vw	−
Tween 20	+	+	+	+
Tween 40	+	+	+	+
Tween 60	+	+	+	+
Tween 80	+	+	+	+
Culture medium:				
Blood agar	−	−	−	v −
Cetrimide agar	−	−	vw	−
MacConkey agar	−	−	−	v −
TSA + 10 mM KNO_3	+	+	−	v +
Hydrolysis of:				
Casein	v +	+	v +	v −
Starch	v +	+	v +	v +
API 20NE:				
Esculin hydrolysis	−	−	−	v −
Gelatin liquefaction	+	+	v +	+
Nitrate reduction	+	+	v −	v +
PNPG β-galactosidase	v −	+	v +	+
Assimilation of:				
Adipate	v −	−	v +	−
Caprate	−	v −	+	−
Citrate	v −	+	+	+
Malate	+	+	+	v +
Phenylacetate	−	−	v +	−
API ZYM:				
Alkaline phosphatase	+	+	+	v +
Butyrate esterase (C_4)	+	+	+	v −
β-galactosidase	−	−	−	v −
β-glucosidase	−	−	−	v −
Myristate lipase (C_{14})	−	+	+	+
Valine arylamidase	−	v −	v +	v −

overlaid with 1 µL of matrix solution, which consisted of 10 mg α-cyano-4-hydroxycinnamic acid dissolved in 1 mL acetonitrile:trifluoroacetic acid:Milli-Q (50:2.5:47.5) water-solvent. Prior to the analysis, the mass spectrometer was externally calibrated using the Bacterial Test Standard (Bruker Daltonik, Germany). Samples were analyzed automatically using the Bruker Microflex™ LT/SH smart instrument (flexControl version 3.4). The flexAnalysis Batch Process (Bruker Daltonik, Germany) was used to convert the mass spectra into text files that were subsequently used as input files in the BioNumerics 7.6.3 software package (Applied Maths, Belgium). Curve-based data analysis of mass spectra was performed using the Pearson product-moment correlation coefficient and the UPGMA (Unweighted Pair Group method with Arithmetic Mean) cluster algorithm. MALDI-TOF MS analysis revealed that the isolates CBAS 719 [T], CBAS 732 and CBAS 720 displayed similar mass spectra that differed from those of closely related species, confirming their unique taxonomic position (Fig. 3).

The phenotypic, chemotaxonomic and genomic data presented in this study demonstrated that strains CBAS 719 [T], CBAS 732 and CBAS 720 represent a novel species in the genus *Burkholderia* that can be distinguished from its nearest phylogenetic neighbors, both phenotypically and genotypically. This bacterium is closely related to *B. plantarii*, *B. glumae* and *B. gladioli*, which are pathogens of various plants including *Oryza sativa*, *Gladiolus* sp. and *Vanda* sp. [28]. The novel species did not cause disease on sisal and onion, both monocotyledon plant species [10].

Antagonistic activity against plant pathogens

The effect of volatile organic compounds (VOCs) produced by different strains of the newly described *Burkholderia* species on mycelial growth of plant pathogens was evaluated in Petri plates split into two compartments, both containing the MEA medium. A 100-µL aliquot of suspension of each bacterial strain adjusted to $OD_{600} = 0.05$ was spread on one of the compartments and a 5-mm diam mycelial disc of each tested plant pathogen, including *Aspergillus welwitschiae* 131, *Moniliophthora perniciosa* CEPEC/CEPLAC 2421 and *Phytophthora palmivora* CEPEC/CEPLAC 1913, was placed at the centre of opposite compartments in the same plate.

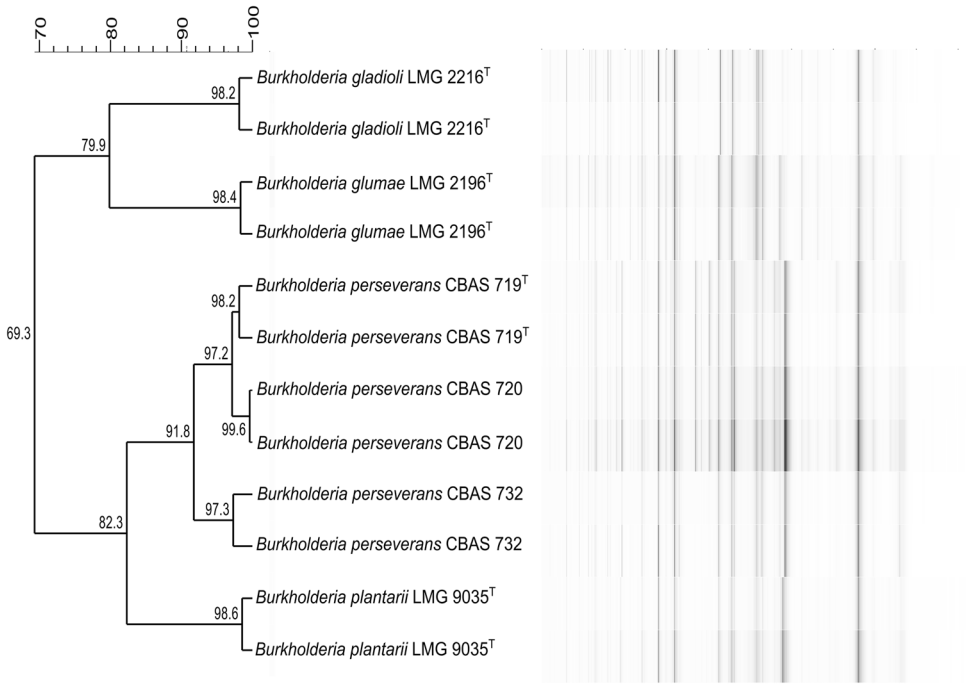

Fig. 3 Curve-based cluster analysis using the Pearson product-moment correlation coefficient and the UPGMA cluster algorithm of mass spectra generated from *B. perseverans* sp. nov. and the type strains of its closest phylogenetic neighbors. The mass spectra were obtained in the MALDI-TOF MS analysis with cell extracts prepared from pelleted bacterial cells

Each combination of bacterial strain and plant pathogen was tested separately in the same experiment and the pathogens without the bacterial treatments served as controls. Plates were sealed with parafilm and incubated at 28 °C for 8 days. Mycelial growth was measured daily and the average inhibition in relation to the control was determined. Analysis of variance and mean separation with Tukey's test at 5% probability were done by using the R software [29]. The experiment was arranged in a completely randomized design with four replicates and was performed two times with similar results. The three different strains of the novel *Burkholderia* species produced VOCs that significantly inhibited mycelial growth of *A. welwitschiae*, *M. perniciosa* and *P. palmivora* (Fig. 4), which represent diverse groups of plant

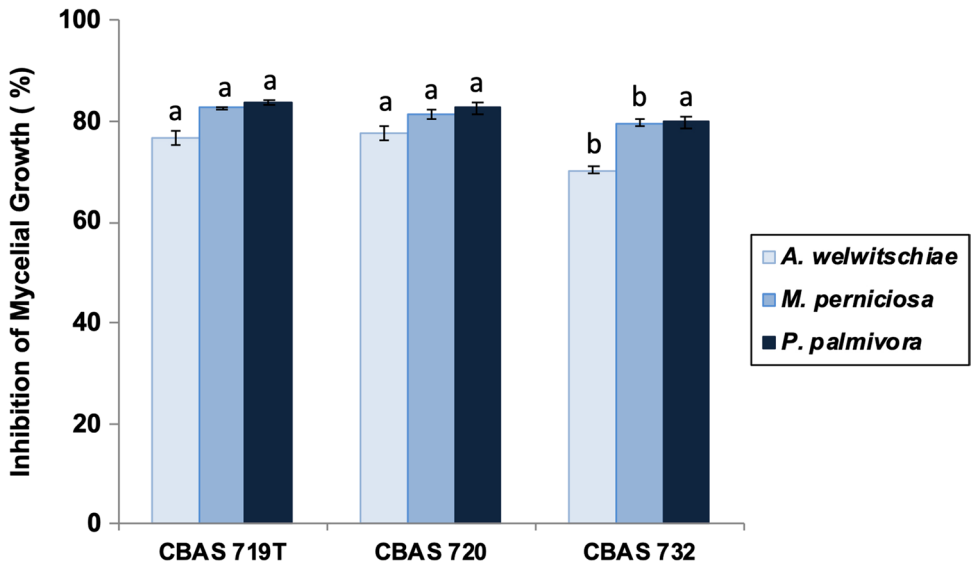

Fig. 4 Inhibition of mycelial growth of diverse plant pathogens by three strains of *B. perseverans* sp. nov. The experiment was done in Petri plates split into two compartments containing the MEA medium and the controls were the pathogens growing without bacterial strains on the other compartment. Plates were incubated for 8 days and the average percentage of inhibition in relation to the respective control was determined. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$). Error bars represent the standard error of the means. Comparisons should be done only among different bacterial strains for the same plant pathogen

pathogens. The in vitro inhibition in relation their respective controls varied from 70 to 78% against *A. welwitschiae* and from 80 to 84% against *M. perniciosa* and *P. palmivora* (Fig. 4).

We have previously shown in in vitro and field experiments that this bacterium can be used as a biological control agent that produces volatile and diffusible organic compounds that are able to inhibit mycelial growth and spore germination of *A. welwitschiae*, which is the main etiological agent of the bole rot disease of sisal [10]. This crop possesses great socio-economic importance since it is cultivated employing familiar labor and Brazil is the main producer [30, 31].

Description of *Burkholderia perseverans* sp. nov.

Burkholderia perseverans sp. nov. (per.se.ve'rans, L. fem. part. pres. as reference to the environment from which this species was isolated, characterized by adverse conditions including low humidity, high temperatures and poor and acidic sandy soils). Gram-negative, aerobic, motile, non-spore-forming rods, about 1.0–3.0 µm long. Colonies were round, with smooth margins, a low convex elevation, and a non-pigmented and translucent appearance, and are 1 mm in diameter after 72 h of growth on TSA at 28 °C. Growth occurred well at 15–37 °C and weakly at 40 °C, at pH 6–7 at 28 °C and with 0–3% (variable; type strain negative) (w/v) NaCl. It grew on MacConkey agar, but lactose was not fermented. It grew on blood agar, but did not produce hemolysis. It grew on medium with tween 20, 40, 60 and 80, and on TSA with 10 mM of KNO_3. It did not grow on cetrimide agar. Casein and starch hydrolysis were strain dependent but the type strain was positive. It has no DNase activity. Catalase and oxidase activities were observed. In API 20NE strips, nitrate was reduced, and glucose, arabinose, mannose, mannitol, N-acetylglucosamine, gluconate and malate were assimilated, but not maltose, phenylacetate, caprate, adipate (variable; type strain negative) or citrate (variable; type strain negative). It is negative for fermentation of glucose, activities of tryptophanase, arginine dihydrolase, urease, β-galactosidase (PNPG) (variable; type strain negative) and hydrolysis of aesculin, but positive for gelatin liquefaction. When tested by using API ZYM strips, activities of the following enzymes were positive: alkaline and acid phospatases, leucyl arylamidase, phosphoamidase, C4 lipase and C8 lipase; activities were negative for C14 lipase, valine and cystine arylamidases, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, α-glucosidase, N-acetyl-β-glucosaminidase, α- mannosidase and α-fucosidase. The most abundant fatty acids were $C_{16:0}$, $C_{18:1}$ ω7c, cyclo-$C_{17:0}$ and summed feature 3 ($C_{16:1}$ ω7c and/or $C_{15:0}$ *iso* 2-OH).

Protologue

The type strain, CBAS 719 ^{T} (=LMG 31557 ^{T} = INN12^T), was isolated from leaf litter samples at Espírito Santo province, Brazil, in 2008. The DNA G + C content of the type strain was 69.01 mol%. The draft genome sequence of type strain is 7.57 Mbp consisting of two chromosomes, which have been deposited in DDBJ/EMBL/GenBank under accession numbers CP045094—CP045093.

Abbreviations ANI: Average nucleotide identity; Bcc: *Burkholderia cepacia* Complex; dDDH: Digital DNA-DNA hybridization; ML: Maximum likelihood; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry

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Declarations

Conflict of interest The authors declare no competing interests.

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