

# *Pseudomonas capsici* sp. nov., a plant-pathogenic bacterium isolated from pepper leaf in Georgia, USA

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## Abstract

Three phytopathogenic bacterial strains (Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3) were isolated from seedlings displaying water-soaked, dark brown-to-black, necrotic lesions on pepper (*Capsicum annuum*) leaves in Georgia, USA. Upon isolation on King's medium B, light cream-coloured colonies were observed and a diffusible fluorescent pigment was visible under ultraviolet light. Analysis of their 16S rRNA gene sequences showed that they belonged to the genus *Pseudomonas*, with the highest similarity to *Pseudomonas cichorii* ATCC 10857<sup>T</sup> (99.7%). The fatty acid analysis revealed that the majority of the fatty acids were summed feature 3 (C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c), C<sub>16:0</sub> and summed feature 8 (C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c). Phylogenomic analyses based on whole genome sequences demonstrated that the pepper strains belonged to the *Pseudomonas syringae* complex with *P. cichorii* as their closest neighbour, and formed a separate monophyletic clade from other species. Between the pepper strains and *P. cichorii*, the average nucleotide identity values were 91.3%. Furthermore, the digital DNA–DNA hybridization values of the pepper strains when compared to their closest relatives, including *P. cichorii*, were 45.2% or less. In addition, biochemical and physiological features were examined in this study and the results indicate that the pepper strains represent a novel *Pseudomonas* species. Therefore, we propose a new species *Pseudomonas capsici* sp. nov., with Pc19-1<sup>T</sup> (=CFBP 8884<sup>T</sup>=LMG 32209<sup>T</sup>) as the type strain. The DNA G+C content of the strain Pc19-1<sup>T</sup> is 58.4 mol%.

The members of *Pseudomonas* species have been isolated from various environmental sources including soil, water, plants and animals [1]. In the past, DNA–DNA hybridization (DDH) was considered the gold standard for prokaryotic species differentiation [2]. However, DDH is laborious and a difficult approach. Sequence analysis of 16S rRNA genes and conserved housekeeping genes are often conducted for characterization of *Pseudomonas* species [3, 4], but not accepted as valid standards for bacterial species delineation. Instead, recent developments of whole-genome sequencing technologies have advanced the sequence-based taxonomy for bacteria [5].

Multiple *Pseudomonas* species and pathovars have been associated with causing diseases on a wide range of plant hosts [6]. Among the *Pseudomonas* species, *Pseudomonas syringae* has been widely studied and taxonomically well-characterized compared with other plant-pathogenic bacterial species. The

multi-locus sequence analysis of housekeeping genes has been used to assign 13 phylogroups and nine genomospecies within the *P. syringae* complex [4, 6]. Due to the advances in sequencing technology, whole-genome sequences (WGS) have been used for classifying *Pseudomonas* species taxonomically [5]. Specifically, multiple pairwise comparative approaches like average nucleotide identity (ANI) and genome-to-genome distance calculations have been used for species differentiation using WGS [7]. Recently, three fluorescent *Pseudomonas* strains were isolated from symptomatic pepper foliage in Georgia, USA. Using a polyphasic taxonomic approach, we provide evidence that these strains represent a novel *Pseudomonas* species. The pepper strains Pc19-1, Pc19-2 and Pc19-3 are being proposed as *Pseudomonas capsici* sp. nov.

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**Keywords:** average nucleotide identity; novel species; pepper; *Pseudomonas capsici*.

**Abbreviations:** AIC, Akaike Information Criterion; ANI, average nucleotide identity; ANIb, ANI based on BLAST; dDDH, digital DDH; DDH, DNA–DNA hybridization; LIN, Life Identification Number; NCBI, National Center for Biotechnology Information; WGS, whole-genome sequence.

The GenBank/EMBL/DDBJ accession numbers for the genomes of strains Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3 are JAFGZD000000000, JAFGZE000000000 and JAFGZF000000000, respectively. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences of strains Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3 are MW583591, MW583592 and MW583593, respectively.

Four supplementary figures are available with the online version of this article.

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**Table 1.** Genomic relationship between strain Pc19-1<sup>T</sup> and the type strains of closely related *Pseudomonas* species as well as other *Pseudomonas capsici* sp. nov. strains

The 16S rRNA gene sequences were retrieved from GenBank by similarity searches using the strain Pc19-1<sup>T</sup> sequence as a query. ANib and dDDH values were calculated using JSpeciesWS version 1.2.1 [17] and the Genome-to-Genome Distance Calculator 2.1 (formula 2) [18], respectively.

Species	16S rRNA gene sequence similarity (%)	dDDH (%)	ANib (%)
<i>Pseudomonas capsici</i> Pc19-2	100	100	100
<i>Pseudomonas capsici</i> Pc19-3	100	100	100
<i>Pseudomonas cichorii</i> ATCC 10857 <sup>T</sup>	99.7	45.2	91.3
<i>Pseudomonas viridiflava</i> DSM 6694 <sup>T</sup>	97.9	25	80.6
<i>Pseudomonas meliae</i> CFBP 3225 <sup>T</sup>	98.3	24.8	80.8
<i>Pseudomonas asturiensis</i> LMG 26898 <sup>T</sup>	97.6	24.8	80.6
<i>Pseudomonas cannabina</i> ICMP 2823 <sup>T</sup>	98.0	24.8	80.6
<i>Pseudomonas floridensis</i> GEV388 <sup>T</sup>	98.4	24.8	80.5
<i>Pseudomonas amygdali</i> CFBP 3205 <sup>T</sup>	96.9	24.7	80.5
<i>Pseudomonas congelans</i> DSM 14939 <sup>T</sup>	98.2	24.6	80.2
<i>Pseudomonas syringae</i> KCTC 12500 <sup>T</sup>	98.2	24.6	80.1
<i>Pseudomonas ovata</i> F51T <sup>T</sup>	98.8	23.3	78.4
<i>Pseudomonas chlororaphis</i> LMG 5004 <sup>T</sup>	98.3	23.2	77.6
<i>Pseudomonas chlororaphis</i> subsp. <i>aureofaciens</i> NBRC 3521 <sup>T</sup>	98.3	23.2	77.4
<i>Pseudomonas mediterranea</i> CFBP 5447 <sup>T</sup>	98.2	23.1	76.8
<i>Pseudomonas chlororaphis</i> subsp. <i>aurantiaca</i> DSM 19603 <sup>T</sup>	98.3	23	77.5
<i>Pseudomonas brassicacearum</i> LMG 21623 <sup>T</sup>	97.6	23	76.9
<i>Pseudomonas chlororaphis</i> subsp. <i>piscium</i> DSM 21509 <sup>T</sup>	98.2	22.9	77.4
<i>Pseudomonas viciae</i> 11K1 <sup>T</sup>	98.0	22.8	76.8

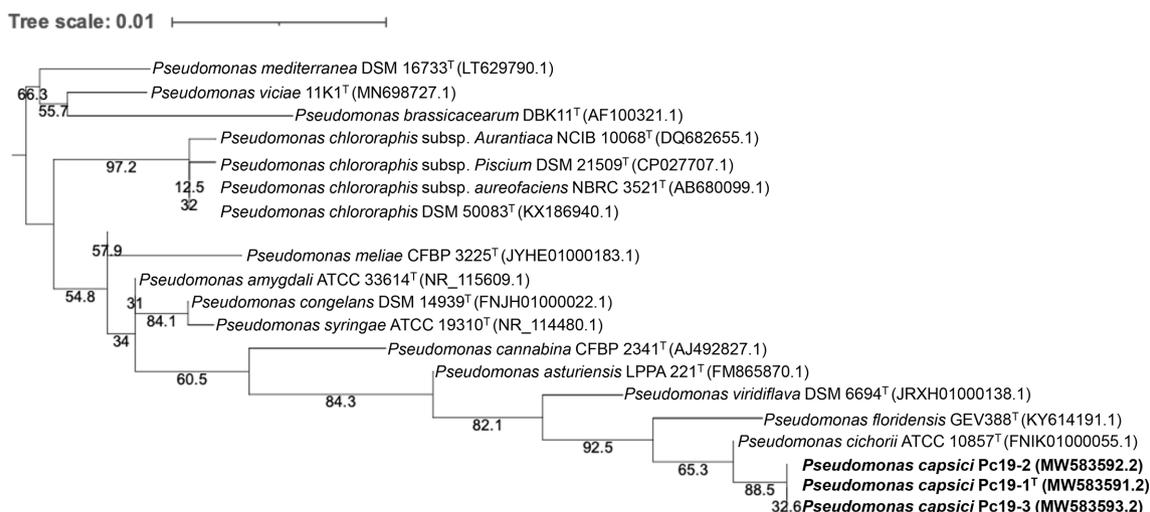
## ISOLATION AND ECOLOGY

Leaf blight symptoms were observed in pepper seedlings in greenhouses in Georgia, USA in 2019. Foliar symptoms included water-soaked, dark brown-to-black, irregularly shaped lesions (Fig. S1, available in the online version of this article). Three fluorescent *Pseudomonas* strains were isolated from the symptomatic tissue of pepper leaves on King's medium B. Pathogenicity of isolated bacterial strains was confirmed by leaf infiltration of pepper foliage (cv. Aristotle) using a syringe with bacterial suspensions containing approximately  $1 \times 10^6$  c.f.u. ml<sup>-1</sup> [8]. Disease symptoms similar to the original natural infections were observed 48 h after inoculation by strains Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3, while *P. cichorii* type strain NCPPB943<sup>T</sup> did not cause necrosis on pepper leaf (Fig. S1). The standard LOPAT tests [9], which consist of determining levan production on 5% sucrose medium, oxidase activity, potato soft rot ability, arginine dihydrolase assay and tobacco hypersensitivity, were conducted for preliminary characterization. The three pepper strains were positive for the oxidase test, caused potato rot (Fig. S2), and showed a hypersensitive reaction on tobacco. However, they were unable to produce levan and were negative for the arginine-dihydrolase assay. These characteristics are unique

when compared to the LOPAT profiles of the fluorescent plant pathogenic *Pseudomonas* species reported by Lelliott *et al.* [9], but closely matched with *P. cichorii* except for the potato rot test.

## 16S rRNA GENE PHYLOGENY

Phylogenetic analysis was conducted using 16S rRNA gene sequences. The 16S rRNA genes of the three pepper strains was amplified using primers 27F and 1492R, and the resulting products were sequenced with primers 27F and 1492R using Sanger sequencing by Eurofins Genomics (Louisville, KY). The partial 16S rRNA gene sequences of strains Pc19-1, Pc19-2 and Pc19-3 were deposited in GenBank (accession numbers: MW583591, MW583592 and MW583593). The sequences were compared with sequences of the type strains of 17 closely related *Pseudomonas* species downloaded from NCBI. Comparative analysis revealed that the three pepper strains had identical partial 16S rRNA gene sequences and their sequence identities with *P. cichorii* type strain ATCC 10857<sup>T</sup> were all 99.7% (Table 1). The next species was *Pseudomonas ovata* F51T<sup>T</sup> with 98.8% 16S rRNA gene sequence identities. The sequences were aligned using MAFFT (version



**Fig. 1.** Phylogenetic relationships based on partial gene sequences of 16S rRNA between *Pseudomonas capsici* sp. nov. strains and closely related *Pseudomonas* species listed in Table 1. The 16S rRNA gene sequences (1266 nucleotides) were aligned using MAFFT (version 7.294b) [10]. The alignment was used to construct a phylogenetic tree using the PHYL package with the maximum-likelihood method and with the best substitution model estimated by jmodelTest version 2.1.10 [12]. The clade including *Pseudomonas viciae*, *Pseudomonas brassicacearum* and *Pseudomonas mediterranea* was used for outgroup rooting. Numbers at nodes represent bootstrap values from 1000 replicates. Bar, 1 nt substitution per 100 nt. GenBank accession numbers are shown within parentheses along with the strain, with <sup>T</sup> indicating type strains.

7.294b) [10] within Geneious Prime 2019. A maximum-likelihood phylogenetic tree using 16S rRNA gene sequences (1266 nucleotides) was reconstructed using the PHYL package [11]. The model for phylogenetic analysis was determined using the Akaike Information Criterion (AIC) statistic within jModelTest version 2.1.10 [12]. Based on the AIC, a TIM3 substitution model with invariable sites (+I) and rate variation among sites (+G) was used [13]. The robustness of the topology was estimated using 1000 bootstrap replicates. Phylogenetically, the strains were closely related to *P. cichorii* (Fig. 1).

## GENOME FEATURES

The whole genomes of strains Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3 were sequenced for taxonomic status analysis. DNA was extracted using the EZNA Bacterial DNA Kit (Omega Bio-tek). The genomic libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit for Illumina and were sequenced using an Illumina Novaseq 6000 platform. The raw sequences were filtered using fastp 0.20.0 [14] and then assembled using SPAdes version 3.14 [15]. The assembled sequences were deposited at GenBank (accession numbers: JAFGZD000000000, JAFGZE000000000 and JAFGF000000000). They were also uploaded to the Life Identification Number (LIN) platform [16]. The LINs for Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3 are ‘50,1,0,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0’, ‘50,1,0,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1’ and ‘50,1,0,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,2’, respectively. The N50 values for strains Pc19-1<sup>T</sup> (332309 bp), Pc19-2 (241172 bp) and Pc19-3 (205290 bp) were determined. The contig numbers for the assembled sequences of Pc19-1<sup>T</sup>, Pc19-2 and Pc19-3 are 59, 55

and 59, respectively. The genome assembly of strain Pc19-1<sup>T</sup> yielded a genome size of 5843696 nucleotides and its G+C content was 58.4 mol%.

The taxonomic position of the three pepper strains at the species level was determined by comparing the ANI values and digital DNA–DNA hybridization (dDDH) values between the pepper strains and 17 closely related *Pseudomonas* species. Pairwise ANI values based on BLAST (ANiB) were calculated between Pc19-1<sup>T</sup> and phylogenetically closely related *Pseudomonas* species using jSpeciesWS version 1.2.1 [17]. The dDDH values were calculated using formula 2 of the Genome-to-Genome distance calculator 2.1 [18] using the Type Strain Genomic Server (TYGS) [19]. The calculated ANiB and dDDH values are shown in Table 1. The highest ANiB value for Pc19-1<sup>T</sup> was observed with *P. cichorii* ATCC10857<sup>T</sup> at 91.3% (Table 1), which is lower than the threshold value of 95% considered as the standard ANI value for species differentiation [20, 21]. Moreover, the dDDH value estimated for Pc19-1<sup>T</sup> and the *P. cichorii* type strain was 45.2%, which is below the 70% threshold for prokaryotic species delineation [20, 21].

The phylogenomic analysis was carried out using the TYGS [19]. Pairwise genome comparisons were conducted using the genome BLAST distance phylogeny (GBDP) approach [18], and intergenomic distances were inferred under the algorithm ‘trimming’ and distance formula  $d_s$ . The resultant intergenomic distances were used to generate a minimum-evolution tree via FastME 2.1.6.1 [22] including subtree pruning and regrafting post processing. Branch supports were inferred from 100 pseudo-bootstrap repetitions. The three



**Table 2.** Genomic relationship of strains listed under *Pseudomonas cichorii* in NCBI compared to strain Pc19-1<sup>T</sup> and *P. cichorii* ATCC10857<sup>T</sup>

ANIb and dDDH values were calculated using JSpeciesWS version 1.2.1 [17] and the Genome-to-Genome Distance Calculator 2.1 (formula 2) [18], respectively. ANIb values larger than 95% are in bold. dDDH values larger than 70% are in bold.

Strain	ANIb (%)		dDDH (%)		Species status	Reference
	<i>P. capsici</i> 19-1 <sup>T</sup>	<i>P. cichorii</i> ATCC10857 <sup>T</sup>	<i>P. capsici</i> 19-1 <sup>T</sup>	<i>P. cichorii</i> ATCC10857 <sup>T</sup>		
MAFF302698	<b>98.5</b>	91.3	<b>88.3</b>	45.2	<i>P. capsici</i>	[30]
481	<b>98.5</b>	91.3	<b>87.9</b>	45	<i>P. capsici</i>	N/A
Ku1409-10-1	<b>98.4</b>	91.3	<b>88.1</b>	45.2	<i>P. capsici</i>	[30]
NB15027	<b>98.4</b>	91.3	<b>87.9</b>	45.2	<i>P. capsici</i>	[30]
482	<b>98.4</b>	91.3	<b>87.8</b>	45.1	<i>P. capsici</i>	N/A
136	<b>98.4</b>	91.3	<b>87.8</b>	45.3	<i>P. capsici</i>	N/A
ICMP1649	<b>98.4</b>	91.3	<b>88.4</b>	45.3	<i>P. capsici</i>	N/A
474	<b>98.4</b>	91.3	<b>87.8</b>	45.3	<i>P. capsici</i>	N/A
S-2-2-1	<b>98.4</b>	91.3	<b>87.7</b>	45.2	<i>P. capsici</i>	[30]
473	<b>97.2</b>	91.2	<b>77.1</b>	45.3	<i>P. capsici</i>	N/A
Pcic4	92.1	94.0	48.4	57.6	Potential new species	N/A
Ku1408-5-5	92.1	94.5	48.2	59.1	Potential new species	[30]
REF	91.4	<b>99.9</b>	45.3	<b>99.8</b>	<i>P. cichorii</i>	N/A
JBC1	91.4	<b>99.9</b>	45.2	<b>99.8</b>	<i>P. cichorii</i>	[31]
MAFF301184	91.4	<b>99.0</b>	45.1	<b>92.2</b>	<i>P. cichorii</i>	[30]
ICMP6917	91.4	<b>99.3</b>	45.1	<b>94.4</b>	<i>P. cichorii</i>	N/A
MAFF302096	89.0	91.4	38.1	45.2	Potential new species	[30]
MAFF301764	88.9	91.0	37.8	43.9	Potential new species	[30]
ICMP3353	86.8	86.6	32.7	32.6	Potential new species	N/A

acid profiles of the pepper strains were closely related to those of *P. cichorii*.

In addition, analyses of polar lipids and respiratory quinones were carried out by the Identification Service of the DSMZ (Braunschweig, Germany) based on previously described methods [24–27]. The major polar lipids found in Pc19-1<sup>T</sup> were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol (Fig. S4), which is consistent with species of the genus *Pseudomonas* [28]. Minor amounts of phosphatidylcholine, aminophospholipid and one unidentified lipid were also detected (Fig. S4). The major respiratory quinone was Q9 (96.9%), which is consistent with other *Pseudomonas* species. Additional respiratory quinones detected were Q8 (1.7%) and Q10 (1.4%).

Considering the results of polyphasic analyses based on phenotypic characteristics, metabolic reactions, fatty acid composition, phylogenetic studies and whole-genome sequence comparisons, the three strains isolated from pepper in Georgia represent a novel species, and the name *Pseudomonas capsici* sp. nov. is proposed.

## DESCRIPTION OF *PSEUDOMONAS CAPSICI* SP. NOV.

*Pseudomonas capsici* (cap'si.ci. N.L. neut. gen n. *capsici*, referring to *Capsicum*, the genus name of pepper).

The colonies are light cream, opaque, round and 1.0–2.0 mm diameter after incubation at 28 °C for 24 h on nutrient agar medium. The cells are Gram-negative, aerobic, motile with multiple polar flagella and rod-shaped (2.4 µm long and 0.7 µm wide). Strains are levan-negative, oxidase-positive, positive for potato rot activity and arginine-dihydrolase-negative, and induce hypersensitive reactions on tobacco. The cells are fluorescent on King's B medium under ultraviolet light. Cell growth occurs at 4–37 °C, with optimum growth observed between 28 and 30 °C. The bacterium grows at pH 5–6 and with 1–4% NaCl. The fatty acids comprise summed feature 3 (C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c), C<sub>16:0</sub>, summed feature 8 (C<sub>18:1</sub> ω7c and/or C<sub>18:1</sub> ω6c), C<sub>12:0</sub>, C<sub>12:0</sub> 3-OH, C<sub>10:0</sub> 3-OH, C<sub>12:0</sub> 2-OH, 11-methyl C<sub>18:1</sub> ω7c and C<sub>18:0</sub>. The bacterium has diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as major

**Table 3.** Selected phenotypic characteristics and substrate-utilization profiles of strain Pc19-1<sup>T</sup> and representative strains of closely related *Pseudomonas* species

Strains: 1, Pc19-1<sup>T</sup>; 2, *P. cichorii* ATCC 10857<sup>T</sup>; 3, *P. floridensis* GEV388<sup>T</sup>; 4, *P. viridiflava* ICMP 2848<sup>T</sup>; 5, *P. asturiensis* LMG 26898<sup>T</sup>; 6, *P. syringae* LMG 1247<sup>T</sup>. All strains were negative for the production of arginine dihydrolase, and induced a hypersensitive reaction on tobacco. Data of columns 2–6 taken from Timilsina et al. [32]. +, Positive reaction; –, negative reaction; w, weakly positive reaction.

Characteristic	1*	2	3	4	5	6
Levan formation from sucrose	–	–	–	–	–	+
Oxidase	+	+	–	–	–	–
Potato rot	+	–	+	+	–	–
Assimilation of (Biolog GEN III):						
D-Sorbitol	–	–	+	+	–	+
L-Galactonic acid	–	–	+	–	+	w
D-Fucose	+	–	w	+	+	+
Sucrose	–	–	–	–	–	+
Troleandomycin	+	–	+	–	w	w

\**Pseudomonas capsici* strains Pc19-2 and Pc19-3 gave identical reactions for these tests as Pc19-1<sup>T</sup>.

polar lipids. Biolog GEN III MicroPlate assays show that Pc19-1<sup>T</sup> can utilize α-D-glucose, D-mannose, D-mannitol, methyl pyruvate, γ-amino-butyric acid, D-fructose, D-arabitol, L-alanine, D-galactose, myo-inositol, D-gluconic acid, L-lactic acid, glycerol, L-aspartic acid, citric acid,

**Table 4.** Cellular fatty acid content (%) of *Pseudomonas capsici* sp. nov. strains and related *Pseudomonas* species

Strains: 1, Pc19-1<sup>T</sup>; 2, Pc19-2; 3, Pc19-3; 4, *Pseudomonas cichorii* DSM 50259<sup>T</sup>; 5, *Pseudomonas floridensis* GEV388<sup>T</sup>.

Fatty acid	1	2	3	4*	5†
C <sub>10:0</sub> 3-OH	3.5	3.6	3.6	2.7	3.6
C <sub>12:0</sub>	4.9	4.8	4.9	4.1	4.7
C <sub>12:0</sub> 2-OH	2.7	2.7	2.6	2.7	2.8
C <sub>12:0</sub> 3-OH	4.0	4.1	4.1	3.8	4.2
C <sub>16:0</sub>	24.6	24.4	24.3	23.9	28.1
C <sub>18:0</sub>	1.0	1.0	1.0	1.1	0.6
11-methyl C <sub>18:1</sub> ω7c	1.1	1.1	1.2	TR‡	0.8
Summed feature 3§	36.2	35.8	36.1	36.9	37.0
Summed feature 8	22.1	21.9	21.6	23.3	16.5

\*Data taken from Burr et al. [33].

†Data taken from Timilsina et al. [32].

‡TR, trace (<1% of total).

§Summed feature 3 comprises C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c.

||Summed feature 8 comprises C<sub>18:1</sub> ω7c and/or C<sub>18:1</sub> ω6c.

D-fucose, L-glutamic acid, glucuronamide, α-keto-glutaric acid, L-fucose, D-fructose-6-PO<sub>4</sub>, mucic acid, D-malic acid, propionic acid, D-aspartic acid, L-pyroglutamic acid, quinic acid, L-malic acid, acetic acid, inosine, L-serine and D-saccharic acid. Strain Pc19-1<sup>T</sup> is able to grow in the presence of 1% sodium lactate, troleandomycin, lincomycin, vancomycin, nalidixic acid, aztreonam, fusidic acid, rifamycin SV, guanidine HC, tetrazolium violet, lithium chloride, D-serine, niaproof 4, tetrazolium blue, potassium tellurite and sodium bromate. Using API 20 NE assays, the bacterium assimilates glucose, arabinose, mannose, mannitol, gluconate, caprate, malate and citrate, and is positive for nitrate reduction to nitrite, urease and β-glucosidase, but negative for indole production, arginine dihydrolase, gelatin hydrolysis, β-galactosidase, assimilation of N-acetyl-glucosamine, maltose, adipate and phenylacetate. The type strain, Pc19-1<sup>T</sup> (=LMG 32209<sup>T</sup>=CFBP 8884<sup>T</sup>), was isolated from symptomatic pepper foliage in Georgia, USA in 2019. The genomic DNA G+C content of the type strain is 58.4 mol%.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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