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TAXONOMIC DESCRIPTION

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Pseudomonas uvaldensis sp. nov., a bacterial pathogen causing onion bulb rot

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Abstract

A Gram-stain-negative, aerobic and non-spore-forming bacterial strain, designated 20TX0172^T, was isolated from a rotting onion bulb in Texas, USA. The results of phylogenetic analysis based on the 16S rRNA sequence indicated that the novel strain represented a member of the genus *Pseudomonas* and had the greatest sequence similarities with *Pseudomonas kilonensis* 520-20^T (99.3%), *Pseudomonas corrugata* CFBP 2431^T (99.2%), and *Pseudomonas viciae* 11K1^T (99.2%) but the 16S rRNA phylogenetic tree displayed a monophyletic clade with *Pseudomonas mediterranea* CFBP 5447^T. In the phylogenetic trees based on sequences of four housekeeping genes (*gap1*, *gltA*, *gyrB* and *rpoD*), the novel strain formed a separate branch, indicating that the strain was distinct phylogenetically from known species of the genus *Pseudomonas*. The genome-sequence-derived average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between the novel isolate and *P. mediterranea* DSM 16733^T were 86.7 and 32.7%, respectively. These values were below the accepted species cutoff threshold of 96% ANI and 70% dDDH, affirming that the strain represented a novel species. The genome size of the novel species was 5.98 Mbp with a DNA G+C content of 60.8 mol%. On the basis of phenotypic and genotypic characteristics, strain 20TX0172^T represents a novel species of the genus *Pseudomonas*. The name *Pseudomonas uvaldensis* sp. nov. is proposed. The type strain is 20TX0172^T (=NCIMB 15426^T=CIP 112022^T).

Species of the genus *Pseudomonas* are Gram-negative, rod-shaped bacteria, motile with one or multiple flagella, non-spore-forming and obligate aerobes [1]. This is one of the most diverse and complex bacterial genera with one of the largest numbers of species among the Gram-negative bacteria [2]. The genus is ubiquitous and cosmopolitan, occurring on all continents, including Antarctica [3–5]. The known onion pathogens of this genus are *Pseudomonas aeruginosa*, *Pseudomonas viridiflava*, *Pseudomonas marginalis*, *Pseudomonas syringae*, *Pseudomonas* coronafaciens, *Pseudomonas allii*, *Pseudomonas kitaguniensis* and *Pseudomonas allivorans* [6–12]. During the mid-1900s, there were more than 800 species assigned to this genus. Many of these species have been reclassified into other genera with the advancement of DNA-based taxonomic tools [1, 13, 14]. Genomic analyses using average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) are robust tools that provide nucleic acid fingerprinting to identify and assign novel prokaryotic species [15, 16]. The number of species of the genus *Pseudomonas* with validly published names has increased from 144 [2] in 2015 to over 250 species at the time of writing (LPSN, https://lpsn.dsmz.de) [17, 18].

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Keywords: onion; average nucleotide identity; digital DNA-DNA hybridization; fatty acid methyl ester; whole genome sequence; 16S rRNA; novel species; *Pseudomonas*.
Abbreviations: ANI, Average nucleotide identity; ANIb, ANI based on BLAST; dDDH, digital DNA–DNA hybridization; FAME, fatty acid methyl ester.
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The GenBank accession number for the genome of strain 20TX0172^T is JAIWKS000000000. The GenBank accession number for the 16S rRNA sequence of strain 20TX0172^T is OK275103.
Eight supplementary figures and one supplementary table are available with the online version of this article.

ISOLATION AND ECOLOGY

Strain 20TX0172^T was isolated from symptomatic tissue of a rotting onion bulb collected in Texas, USA (Fig. S1, available in the online version of this article). For isolation, a 5-mm-diameter piece of bulb tissue was sampled along the margin of the symptomatic tissue, washed with sterile water, and macerated in 100 µl sterile water. The resulting suspension was streaked on nutrient agar (NA) medium. A single colony was purified on NA and incubated at 25 °C. After successive culturing of single colonies, the pure culture was stored at −80 °C in 15% aqueous glycerol (v/v). The isolate was tested for pathogenicity by inoculating the detached fleshy scale from a red onion bulb (Fig. S2) with a bacterial suspension, by injecting a bacterial suspension into yellow onion bulbs (Fig. S3), and inoculating the foliage (Fig. S4) of 8 week-old plants of yellow onion cv. Ranchero with a bacterial suspension [12, 19]. For the scale assay, a 10 µl suspension (10^8 c.f.u. ml^{-1}) of the bacterium was placed on the scale (approximately 3×4 cm) after making a wound at the centre of the scale with a sterile needle, and the scale was incubated at 25 °C for 4 days. For the bulb assay, a 0.5 ml suspension (10^8 c.f.u. ml^{-1}) of the bacterium was injected into the upper shoulder of the bulb, and the bulb was incubated at 25 °C for 12 days. For the foliar assay, a 20 µl suspension (10^8 c.f.u. ml^{-1}) of the bacterium was placed on top of a leaf that was cut approximately 7 cm from the tip, and the plant was incubated at 25 °C for 5 days. Each assay was conducted with three replicates and the experiment was repeated twice. A known pathogenic strain of *Pseudomonas viridiflava* (PV 03–2) was used as a positive control, whereas the sterile PBS solution was used as a negative control [20].

On all the tests, the strain showed a pathogenic response, causing necrosis of fleshy scale, whole bulb and foliage in onion. Two isolates with similar morphology were obtained from each of the three pathogenicity tests. Upon testing these re-isolated daughter strains further on detached scales, whole bulb and foliage of onion, they produced disease symptoms identical to those for the original strain. The genus identity of the three representative daughter isolates (20TX0172 b from fleshy scale, 20TX0172 c from bulb, and 20TX0172 d from foliage) was confirmed as being identical to that of the original isolate used for the pathogenicity tests by sequencing and pairwise alignment of the 16S rRNA gene. The GenBank accession numbers of the three daughter isolates are OL828720, OL863119, and OL866138. On the basis of the results of the phenotypic, genotypic and phylogenetic characterizations, strain 20TX0172^T is proposed to represent a novel species of the genus *Pseudomonas*.

16S rRNA PHYLOGENY

Genomic DNA of 20TX0172^T for amplification and sequencing was extracted using the DNeasy Power Soil kit (Qiagen) and stored at −20 °C. The universal primers 27F (5′– AGAGTTTGATCMTGGCTCAG –3′), 1492R (5′– CGGTTACCTTGTTACGACTT –3′), and 534R (5′– CGGTTACCTTGTTACGACTT –3′) were used to determine the sequence of the 16S rRNA gene [21–24]. Sequences from three primers were trimmed and assembled to make a consensus sequence of 1440 nucleotides (nt) using Geneious Prime 2020 (https://www.geneious.com/). The partial 16S rRNA gene sequence of 20TX0172^T was deposited in GenBank (OK275103). The sequence was compared with those of 37 type strains of closely related species of the genus *Pseudomonas* downloaded from GenBank that had more than 98% sequence similarity [25] (Table 1). The test strain 20TX0172^T had the greatest sequence similarity with that of *P. kilonensis* 520-20^T (99.3%), followed by *P. corrugata* CFBP 2431^T (99.2%), and *P. viciae* 11K1^T (99.2%). MUSCLE version 3.8.425 [26] was used to align the 16S rRNA gene sequences (1392 nt), from which a maximum-likelihood phylogenetic tree was reconstructed using the default Tamura–Nei model in MEGA version 10.2.6 [27], with 1000 bootstrap replicates. According to the phylogenetic tree (Fig. 1), the novel species formed a monophyletic clade with *P. mediterranea* CFBP 5447^T, supported with an 81% bootstrap value.

GENOME FEATURES

The genome of the novel species was sequenced and assembled at CD Genomics (Shirley, NY, USA). Pair-end sequencing with an average sequencing depth of 100× was performed using the NovaSeq 6000 platform (Illumina). The genome was compiled after trimming and assembling sequences from the raw fastq files using the default settings of Unicycler version 0.4.8 [28] and SPAdes version 3.13.0 [29]. The resulting final genome assembly was deposited in GenBank (JAIWKS000000000). The genome was annotated functionally using PROKKA [30] and the genome size of the novel species was estimated to be 5976542 nt (65 contigs, N50=552539 nt, and the longest contig=783071 nt) with a DNA G+C content of 60.8 mol%. The genome consisted of 5208 coding DNA sequence (CDS), 66 tRNA genes, 2 rRNA (16S and 23S) genes, and 1 transfer–messenger RNA (tmRNA) gene.

Multilocus sequence analysis (MLSA) based on four housekeeping genes (*gap1*, *gltA*, *gyrB* and *rpoD*) was performed on the novel strain and reference strains of the 36 closely related species [31, 32]. The sequence of the novel strain was derived from the genome sequence. The sequences of type strains of the closely related species were downloaded from GenBank. Phylogenetic analysis based on concatenation of the four genes (a total of 2217 nt) was performed as described above using the maximum-likelihood method involving the Tamura–Nei model (Fig. S5), with the resulting tree separating the novel species in a separate clade with *P. corrugata* DSM 7228^T.

The ANI values of the genome sequence of the novel strain with that of *P. mediterranea* DSM 16733^T and the 35 most closely related species were determined using the ANI calculator (https://www.ezbiocloud.net/tools/ani) based on the OrthoANIu

Table 1. Genomic similarities between Pseudomonas *uvaldensis* 20TX0172^T, obtained from a rotting onion bulb in Texas, USA, and the type strains of closely related species of the genus *Pseudomonas* (indicated by ^{T}). The digital DNA–DNA hybridization (dDDH) was calculated using the Type (Strain) Genome Server (formula d4) [35], whereas average nucleotide identity (ANI) values OrthoANIu and ANIb were calculated using ANI calculator [33], and PYANI [34], respectively. The 16S rRNA gene homologies were obtained from GenBank using 20TX0172^T as a query sequence [25]. NG, Genome sequence assembly is not available in public databases

Strain	dDDH (d4, %)	OrthoANIu (%)	ANIb (%)	16S rRNA similarity (%)
Pseudomonas mediterranea DSM 16733^T	32.7	86.7	86.74	98.89
Pseudomonas kilonensis DSM 13647^T	32.6	86.56	86.62	99.27
Pseudomonas brassicacearum JCM 11938^T	32.3	86.5	86.53	98.71
Pseudomonas thivervalensis DSM 13194^T	32	86.21	86.32	99.16
Pseudomonas bijieensis L22-9^T	31.9	86.42	86.32	99.17
Pseudomonas viciae 11K1^T	31.9	86.47	86.32	99.20
Pseudomonas corrugata DSM 7228^T	31.7	86.38	86.23	99.24
Pseudomonas frederiksbergensis FW305-6	26	82.44	82.35	98.56
Pseudomonas silesiensis A3^T	26	82.07	82.25	98.57
Pseudomonas chlororaphis DSM 50083^T	25.8	82.24	82.26	99.06
Pseudomonas migulae NBRC 103157^T	25.8	82.07	81.96	98.62
Pseudomonas lini DSM 16768^T	25.7	81.84	81.97	99.14
Pseudomonas arsenicoxydans CECT 7543^T	25.5	81.83	81.86	98.82
Pseudomonas mandelii DSM 17967^T	25.3	81.82	81.88	98.55
Pseudomonas prosekii LMG 26867^T	25	81.35	81.44	98.92
Pseudomonas veronii DSM 11331^T	24.5	80.9	80.94	98.88
Pseudomonas allii MAFF 301514^T	24.3	80.6	80.71	98.47
Pseudomonas extremaustralis DSM 17835^T	24.3	80.89	80.95	98.61
Pseudomonas extremorientalis LMG 19695^T	24.2	80.47	80.63	98.62
Pseudomonas poae LMG 21465^T	24.2	80.65	80.67	98.53
Pseudomonas trivialis DSM 14937^T	24.2	80.61	80.79	98.59
Pseudomonas fildesensis KG01^T	24.1	80.41	80.65	98.66
Pseudomonas grimontii DSM 17515^T	24.1	80.42	80.64	98.87
Pseudomonas orientalis DSM 17489^T	24.1	80.57	80.62	98.42
Pseudomonas marginalis ICMP 3553^T	23.8	80.34	80.39	98.75
Pseudomonas rhodesiae DSM 14020^T	23.8	80.28	80.53	98.88
Pseudomonas tolaasii CCUG 23369^T	23.8	80.38	80.25	98.39
Pseudomonas antarctica CMS 35^T	23.7	79.9	80.14	98.68
Pseudomonas fluorescens ATCC 13525^T	23.7	80.23	80.32	98.57
Pseudomonas kitaguniensis MAFF 212408^T	23.5	79.82	79.86	98.65
Pseudomonas congelans DSM 14939^T	22.2	77.49	78.09	98.39
Pseudomonas syringae DSM 10604^T	22.2	77.9	78.08	98.24
Pseudomonas savastanoi ICMP 4352^T	22.1	77.39	78.04	98.40
Pseudomonas viridiflava ICMP 2848^T	22.1	77.66	78.16	97.02
Pseudomonas tremae ICMP 9151^T	22	77.27	77.65	98.53
Pseudomonas aeruginosa NCTC10332^T	20.8	76.09	76.78	93.39
Pseudomonas meridiana CMS 38^T	NG	NG	NG	98.61

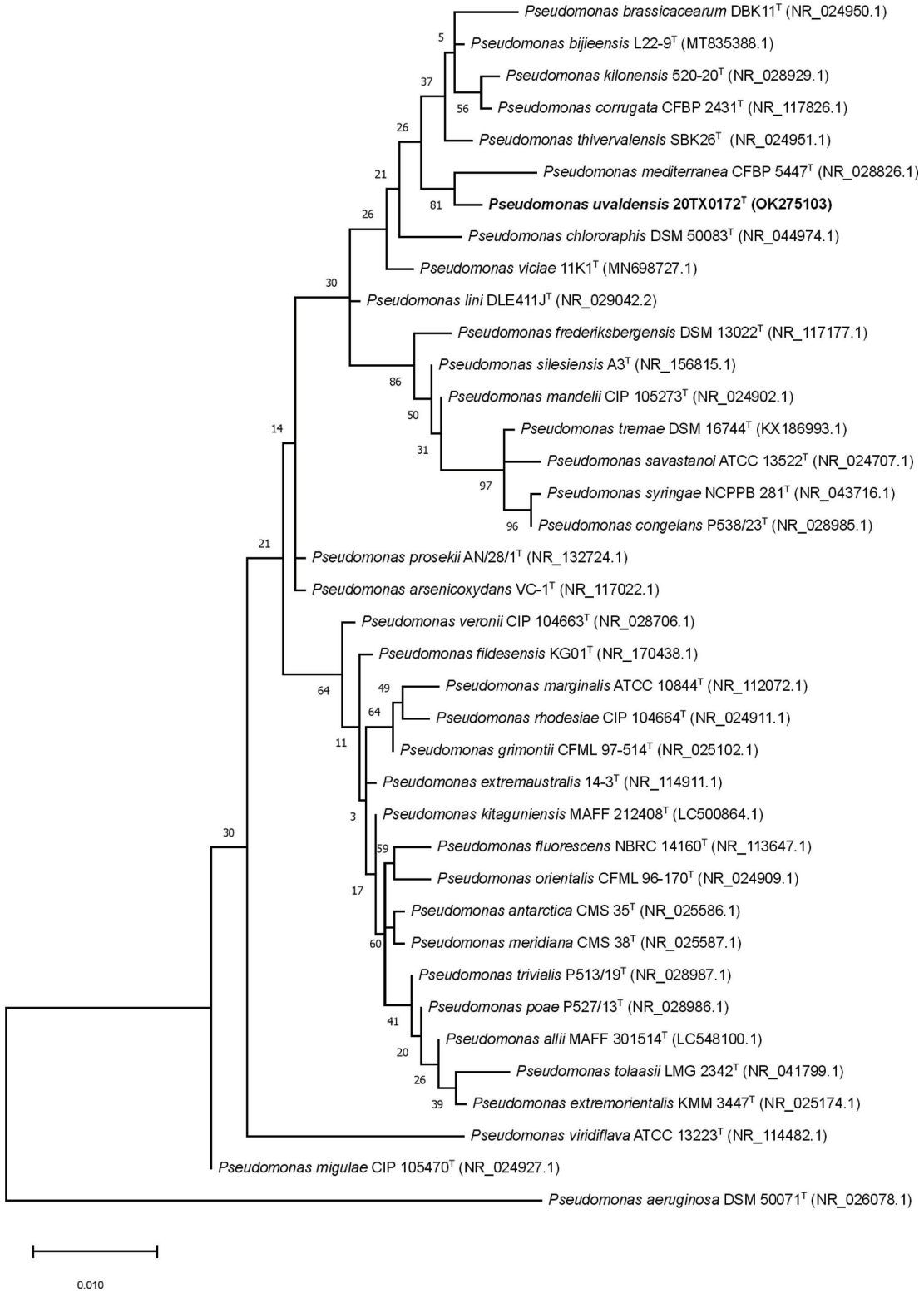

Fig. 1. Maximum-likelihood phylogenetic tree reconstructed using the Tamura–Nei model based on 16S rRNA gene sequences of strain 20TX0172^T of the genus *Pseudomonas* isolated from a rotten onion bulb in Texas, USA, and the sequences of closely related strains of species of the genus *Pseudomonas*. The gene sequences were aligned using MUSCLE V3.8.425, the tree was reconstructed using MEGA V10.2.6, and the text was reformatted using Inkscape V1.1. Bootstrap values at the branching nodes indicate the percentages of 1000 replicates. The scale bar refers to the number of nucleotide substitutions per site. The superscript ^{T} denotes the type strain of the species used, and the GenBank accession numbers are in parentheses.

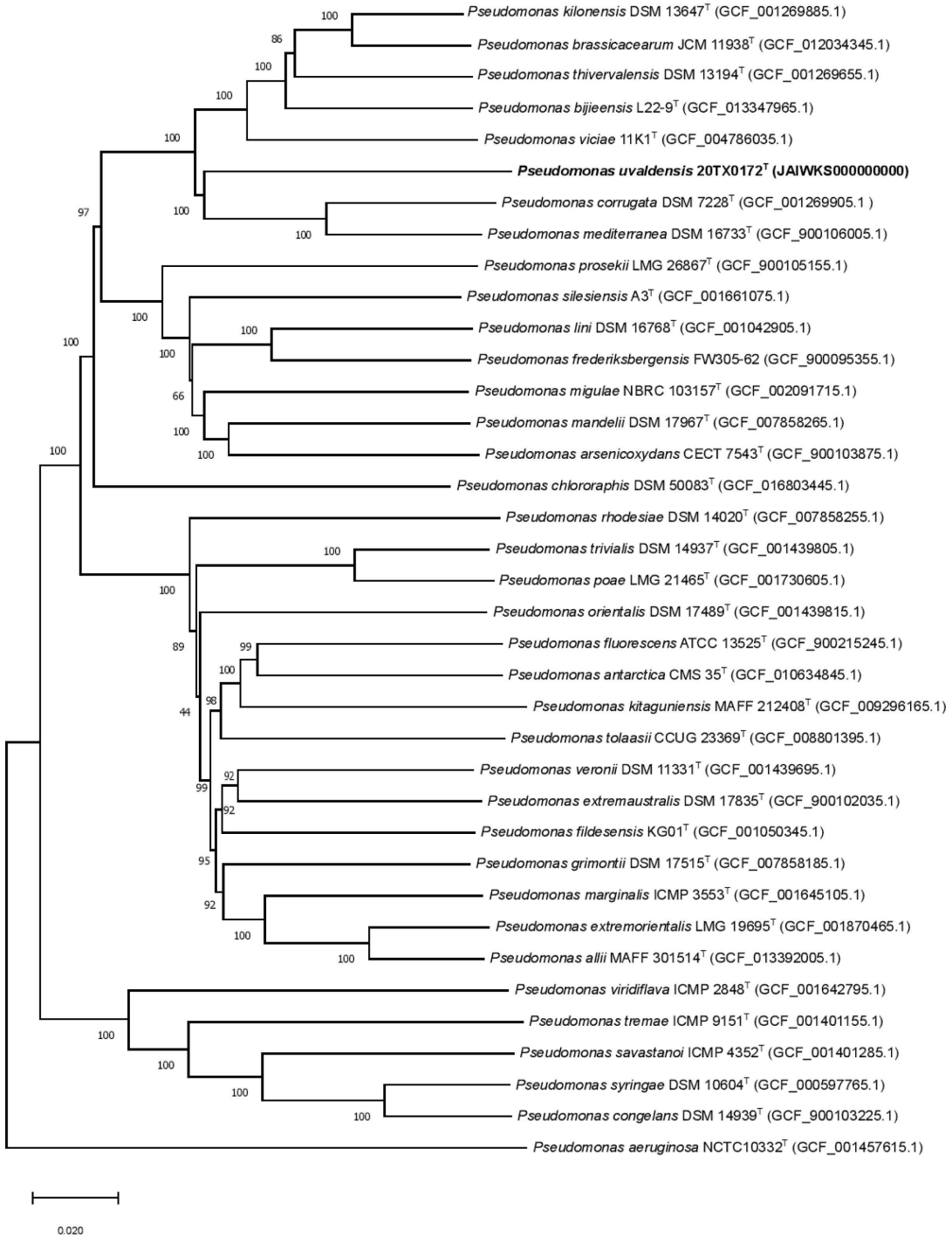

Fig. 2. Phylogenetic tree based on the whole genome sequence of strain 20TX0172^T of the genus *Pseudomonas* isolated from a rotting onion bulb in Texas, USA, and closely related species of the genus *Pseudomonas*. The tree was reconstructed using the TYGS web server, and the text was reformatted using MEGA and Inkscape V1.1. Bootstrap values at the branching nodes indicate the percentage of 1000 replicates. The scale bar refers to the number of nucleotide substitutions per site. The superscript ^{T} denotes the type strain of the species used, and the GenBank accession number of each strain is in parentheses.

algorithm [33], and pairwise ANI values based on BLAST (ANIb) were calculated using Python-based software with the ANIb algorithm – pyANI [34] (Table 1). The dDDH values were calculated using Type (Strain) Genome Server-TYGS (https://tygs.dsmz.de/) [35] (Table 1). The ANI and dDDH values between the novel strain and *P. mediterranea* DSM 16733^T were 86.7 and 32.7%, respectively, less than the cutoff value of 96% ANI and 70% dDDH, respectively, for species determination [15, 16]. A

Table 2. Comparison of selected physiological and phenotypic characteristics of strain 20TX0172^T of the genus *Pseudomonas* obtained from a rotting onion bulb in Texas, USA, and the type strains of the most closely related species of the genus *Pseudomonas*

Strains: 1, 20TX0172^T; 2, *P. corrugata* DSM 7228^T; 3, *P. mediterranea* DSM 16733^T; 4, *P. kilonensis* 520-20^T; 5, *Pseudomonas brassicacearum* CFBP 11706^T; 6, *P. thivervalensis* CFBP 11261^T; 7, *P. bijieensis* L22-9^T; 8, *P. viciae* 11K1^T. +, Positive reaction; –, negative reaction; w, weakly positive reaction; v, variable reaction; ND, no data available. Data in columns 1–3 are from this study and data in columns 4–8 are from [43–47].

Reaction	1	2	3	4	5	6	7	8
KB fluorescence	+	–	–	+	+	+	+	+
Levan	+	–	–	ND	+	+	ND	+
Oxidase	+	+	+	+	+	+	+	+
Pectolytic	–	–	–	ND	–	–	ND	ND
Tobacco hypersensitive response	–	+*	v†	ND	–	–	ND	ND
Catalase	+	+	+	ND	ND	ND	+	+
Biolog:								
D-Aspartic Acid	–	–	+	ND	ND	ND	ND	+
Minocycline	+	–	+	ND	ND	ND	ND	W
Gelatin	–	–	–	+	+	+	ND	W
D-Galacturonic Acid	–	+	+	–	+	V	–	W
L-Galactonic Acid Lactone	–	+	+	ND	ND	ND	+	W
D-Glucuronic Acid	–	+	+	–	+	V	ND	W
p-HydroxyPhenylacetic Acid	–	+	+	ND	ND	ND	+	+
Tween 40	–	–	–	+	+	+	–	–
Formic Acid	–	–	+	+	+	+	–	W
Sodium Bromate	+	W	–	ND	ND	ND	ND	–
API 20 NE:								
Nitrate reduction	+	+	+	–	–	–	–	+
Arginine dihydrolase	+	–	–	–	+	+	W	–
D-mannitol	+	+	+	+	–	–	+	+
N-acetyl-glucosamine	+	+	+	–	+	+	+	–

*Data from [48]
†Data from [49].

phylogenomic tree was created in the TYGS web server and included the genomes of the 36 closely related species downloaded from GenBank (Fig. 2). The Genome BLAST Distance Phylogeny method (GBDP) was used to calculate intergenomic distances. The GBDP method utilized formula d5 [36] and 100 bootstrap replicates were performed for each measurement. The phylogenetic tree was reconstructed using FastME 2.1.4 followed by subtree pruning and regrafting postprocessing. On the basis of this whole genome phylogenetic tree, the novel species formed a separate clade with *P. corrugata* DSM 7228^T and *P. mediterranea* DSM 16733^T, supported with 100% bootstrap value, indicating that this Texas onion strain represents a novel species of the genus *Pseudomonas*.

PHYSIOLOGY AND CHEMOTAXONOMY

Chemotaxonomic analyses stated below were done following the protocols described by Schaad *et al.* [37] and Lelliott *et al.* [38] unless otherwise stated. A 24-h-old culture of 20TX0172^T was tested for the Gram reaction and spore forming capability using the standard Gram staining technique and KOH test [39]. Cultures were grown for 5 days in nutrient agar tubes in the absence of oxygen using a layer of mineral oil to determine the facultatively aerobic status of the strain. Motility was observed by culturing the bacteria in a semi-solid growth motility test medium containing tryptone, sodium chloride and agar. Catalase activity was tested with 3% hydrogen peroxide, and the standard levan production, oxidase production, pectinolytic activity, arginine dihydrolase

Table 3. Comparison of fatty acid composition (percentages) of strain 20TX0172^T of a member of the genus *Pseudomonas* isolated from a rotting onion bulb in Texas, USA, and the type strains of the most closely related species of the genus *Pseudomonas*

Strains: 1, 20TX0172^T; 2, *P. corrugata* NCPPB 2445^T; 3, *P. mediterranea* CFBP 5447^T; 4, *P. kilonensis* DSM 13647^T; 5, *P. brassicacearum* LMG 21623^T; 6, *P. thivervalensis* DSM 13194^T; 7, *P. bijieensis* L22-9^T; 8, *P. viciae* 11K1^T. TR, Trace amount (<1 %); –, not detected. Data for strain 1 is from this study and data for strains 2–8 are from [46] and [47].

Fatty acid	1	2	3	4	5	6	7	8
$C_{10:0}$ 3-OH	TR	5.7	3.9	2.6	3.5	3.9	2.4	3.8
$C_{12:0}$	TR	3.4	4.4	4.3	2	2.7	4.4	3.3
$C_{12:0}$ 2-OH	TR	3.6	3.3	4.4	3	4.5	4.2	4.7
$C_{12:0}$ 3-OH	–	4.7	3.9	4.8	4.1	4.6	4.9	4.7
$C_{16:0}$	38.49	25.2	27.9	26.5	27.3	28.7	25.3	23.4
Summed feature 3*	37.49	27.8	34.2	26.4	36.4	34.6	27.2	22.4
Summed feature 8†	19.13	18.3	20.8	18.7	19	16	19.2	19.5

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.
†Summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

production and tobacco hypersensibility (LOPAT) assays were done [38]. Nutrient agar supplemented with 5% sucrose was used to assess levan production. Oxidase activity was tested using Oxistrips and Oxidrops (Hardy Diagnostics). A pectolytic assay to assess potato soft rotting capability was conducted using flame-sterilized, peeled potato slices, and the tobacco hypersensitivity response was tested *in planta* on a leaf of each of three replicate tobacco plants along with relevant positive and negative control strains of bacteria and buffer.

To determine optimum growth conditions, cultures were incubated on nutrient broth yeast agar (NBYA), yeast extract peptone glucose agar (YPGA), yeast extract dextrose calcium carbonate agar (YDC agar), King's B agar (KB agar), and nutrient agar (NA) media as described by Schaad *et al.* [37] for at least 3 days at 4, 15, 20, 22, 25, 28, 30, 33, 35, 37, 39, 41 and 43 °C. To determine optimal pH for growth, the isolate was incubated in yeast extract peptone glucose (YPG) broth adjusted to a pH of 3, 4, 5, 6, 7, 8, 9 and 10 and the growth was recorded after 7 days at 25 °C. Salt tolerance was determined by culturing the strain on YPGA medium with NaCl concentrations ranging from 0–10%, at intervals of 1% for 7 days at 25 °C. Imaging was performed using cultures that were grown for 24 h at 28 °C in Luria–Bertani (LB) broth and visualized using calibrated magnification with a 15C CCD camera on a 1200Ex transmission electron microscope (TEM; JEOL) operated at 100kV at the Texas A and M Microscopy and Imaging Centre. Biolog GenIII microplates and API 20NE strips (bioMérieux) were utilized to assess various biochemical and physiological characteristics. These tests were carried out using 24-h-old cultures grown in YPGA medium at 30 °C per the manufacturer's instructions. To perform Biolog testing, an IF-A suspension (OD_{600} 0.022) was prepared and dispensed into the GenIII microplates, which were incubated at 30 °C and results read after 24, 48 and 72 h. To conduct API 20NE testing, a bacterial suspension (OD_{600} 0.09) was prepared in 0.85% sterile saline, dispensed into the strips and incubated at 30 °C, and results were read after 24 and 48 h. OD_{600} was measured using a Fluostar Omega microplate reader (BMG Labtech). These two tests were carried out a minimum of three times, and results were compared with the known results for type strains of the seven most closely related species based on the sequence data (Table 2). Among these species, *P. mediterranea* and *P. corrugata* are known pathogens of tomato and pepper [40, 41] but not onion, while the remaining five are not known to be pathogens of any plants. The fatty acid methyl ester (FAME) profile of the novel strain was determined using the Sherlock Microbial Identification System at ESML Analytical (Cinnaminson, NJ, USA) [42].

The cells of 20TX0172^T were Gram-stain-negative and non-spore-forming rods (data not shown), obligately aerobic, motile, catalase-positive, and oxidase-positive. The bacterium produced mucoid, dome-shaped colonies after 24 h at 25 °C on nutrient agar medium supplemented with 5% sucrose, which confirmed the production of levan (Fig. S6). No soft rotting of potato slices was observed after 24 h at 22 °C, indicating negative pectolytic activity. This bacterium showed a negative reaction in the tobacco hypersensitivity test, indicating that it was non-pathogenic to tobacco, and did not elicit hypersensitive response. The cultures grew at temperatures ranging from 4 to 41 °C but did not grow at 43 °C on all the media tested. The optimum temperature for growth was 25–30 °C. While growth was observed at 4 °C, the rate was slow, and it took about 5–7 days to form visible colonies. The bacterium produces a light green, diffusible pigment on KB agar medium, which fluoresced green when exposed to near UV light (360–380 nm) (Fig. S7). On nutrient agar medium, the bacterium produced 0.5 to 1 mm smooth, light-yellow, round, entire, shiny colonies after 24 h of incubation at 28 °C. The colony size increased to 2–3 mm in diameter by 3 days and the pigmentation gradually changed to brown. After 5–7 days, the colonies were amber to reddish-brown in colour, and the culture medium was discoloured likewise. Bacterial growth was observed at a range in pH of 5–10 with optimum growth at a pH of 6–7. The strain grew

on YPGA medium in up to 4% NaCl, while optimum growth was observed at 0–1% NaCl. The bacterial cells were rod-shaped, 1.6–2.6 µm long and 0.6–1.0 µm wide, and motile by means of one to three polar flagella (Fig. S8). Some of the biochemical, Biolog and API 20NE test reactions differentiated the novel strain from the type strains of the closely related species (Table 2). For example, 20TX0172^T was positive for arginine dihydrolase activity in both the Biolog and API tests. The comprehensive results of the Biolog and API 20NE tests for this strain and the closely related strains are provided in Table S1. The major fatty acids in the profile from FAME analysis were $C_{16:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (Table 3). The cellular fatty acid composition of the novel strain was different from that of the type strains of the seven most closely related species, supporting the evidence that this strain represents a novel species of the genus *Pseudomonas*.

DESCRIPTION OF *PSEUDOMONAS UVALDENSIS* SP. NOV.

Pseudomonas uvaldensis (u.val.den'sis N.L. fem. adj. uvaldensis referring to Uvalde, a city located in Texas, USA, which is the reporting Research Centre of the type strain).

Cells of 20TX0172^T are Gram-stain-negative, non-spore-forming, obligately aerobic, oxidase-positive and catalase-positive. They are rod-shaped, 1.6–2.6 µm long and 0.6–1 µm wide, and motile by means of one to three polar flagella. Colonies on nutrient agar are 0.5–1.0 mm wide, smooth, light-yellow, round and entire after 24 h of incubation at 28 °C. In 2–3 days, the colony size increases to 2–3 mm and the pigmentation gradually changes to light amber, then brown to reddish-brown after 5–7 days. The colonies produce a light green, diffusible pigment on KB agar medium, and give a green fluorescence when exposed to near UV light (360–380 nm). The bacterium produces mucoid, dome-shaped colonies with a light yellow, diffusible pigment, confirming levan production. The bacterium has no pectolytic activity, producing no soft rotting of potato slices. It does not produce a hypersensitive response on tobacco. It is positive for arginine dihydrolase activity in both the Biolog and API tests. It can grow at a temperature range of 4–41 °C. The optimum temperature for growth is 25–30 °C. It does not grow at pH 4 or lower and the optimum pH for growth is 6–7. The bacterium can tolerate up to 4% NaCl in growth media, and optimum growth is observed with 0–1% NaCl in YPGA medium.

The type strain, 20TX0172^T (=NCIMB 15426^T=CIP 112022^T) was isolated from a symptomatic onion bulb collected from Texas, USA. The estimated size of the genome is 5976542 nt, with a DNA G+C content of 60.8 mol%. The GenBank accession numbers for the 16S rRNA sequence and draft genome are OK275103 and JAIWKS000000000, respectively.

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

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