**Pseudomonas glycinae** sp. nov. isolated from the soybean rhizosphere

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**Abstract**

Strains MS586ᵀ and MS82, which are aerobic, Gram-negative, rod-shaped, and polar-flagellated bacteria, were isolated from the soybean rhizosphere in Mississippi. Taxonomic positions of MS586ᵀ and MS82 were determined using a polyphasic approach. 16S rRNA gene sequence analyses of the two strains showed high pairwise sequence similarities (>98%) to some *Pseudomonas* species. Analysis of the concatenated 16S rRNA, *rpoB*, *rpoD*, and *gyrB* gene sequences indicated that the strains belonging to the *Pseudomonas koreensis* subgroup (SG) shared the highest similarity with *Pseudomonas kribbensis* strain 46-2ᵀ. Analyses of average nucleotide identity (ANI), genome-to-genome distance, delineated MS586ᵀ and MS82 from other species within the genus *Pseudomonas*. The predominant quinone system of the strain was ubiquinone 9 (Q-9), and the DNA G+C content was 60.48 mol%. The major fatty acids were C₁₆:₀, C₁₇:₀ cyclo, and the summed features 3 and 8 consisting of C₁₆:₁ω7c/C₁₆:₁ω6c and C₁₈:₁ω7c/C₁₈:₁ω6c, respectively. The major polar lipids were phosphatidylglycerol, phosphatidylethanolamine, and diphosphatidylglycerol. Based on these data, it is proposed that strains MS586ᵀ and MS82 represent a novel species within the genus *Pseudomonas*. The proposed name for the new species is *Pseudomonas glycinae*, and the type strain is MS586ᵀ (accession NRRL B-65441 = accession LMG 30275).

**KEYWORDS**

average nucleotide identity, *Pseudomonas glycinae*, rhizosphere, soybean

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1 INTRODUCTION

The genus *Pseudomonas* was first described by Migula (1894). Strains of this genus have been found in natural habitats including plants, soil, animals, and water (Palleroni, 1994). Members of the genus *Pseudomonas* are known to be Gram-negative, rod-shaped, cream-colored, and polar-flagellated. *Pseudomonas* spp. have great metabolic and nutritional versatility. Some strains of *Pseudomonas* spp. play potential roles as bioremediation agents to alleviate various hazardous organic substrates, such as sodium dodecyl sulfate (Furmanczyk, Kaminski, Lipinski, Dziembowski, & Sobczak, 2018). Some strains of *Pseudomonas* spp. promote plant growth directly by facilitating resource acquisition or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development; however, some other strains of *Pseudomonas* can act as pathogens inciting plant diseases (Moore et al., 1996; Oueslati et al., 2019; Ye et al., 2019).
Over 200 species of Pseudomonas are included in the Bacterial Names with Standing in Nomenclature (http://www.bacterio.net). Numerous methods, including physiological, molecular, and phenotypic distinctions (Sneth, Stevens, & Sackin, 1981); 16S rDNA gene sequencing; and multilocus sequence analysis (MLSA) (Pascual, Macián, Arahal, Garay, & Pujaile, 2010), have been used to identify the taxonomic status of Pseudomonas species. With the accumulation of genomic data, the analysis of complete genomes is very useful in Pseudomonas taxonomy (Hesse et al., 2018; Peix, Ramírez-Bahena, & Velázquez, 2018). Average nucleotide identity (ANI) values calculated from genome assemblies have been widely used for the taxonomy of bacteria (Konstantinidis & Tiedje, 2005). ANI evaluates a large number of nucleic acid sequences, including some that evolve quickly and others that evolve slowly, in its calculation and reduces the influence of horizontal gene transfer events or variable evolutionary rates. It has been suggested that species descriptions of bacteria and archaea should include a high-quality genome sequence of at least the type strain as an obligatory requirement (Rosselló-Móra & Amann, 2015). The current metagenome databases have shown evidence for approximately 8000 sequence-discrete natural populations, which is roughly equivalent to species at the 95% ANI level (Rosselló-Móra & Whitman, 2018). Genome-to-genome distance (GGDC 2.0) is another highly effective method for inferring whole-genome distances. GGDC effectively mimics DNA-DNA hybridization for genome-based species delineation and subspecies delineation (Meier-Kolthoff, Auch, Klenk, & Göker, 2013). Therefore, ANI and GGDC are highly effective ways to evaluate the genetic relatedness between genomes. Strains MS586\(^7\) and MS82 were isolated from the rhizosphere soybean plants growing in fields where most plants were infected by the charcoal rot pathogen Macrophomina phaseolina. Plate bioassay indicated both strains MS586\(^7\) and MS82 exhibited striking antimicrobial activity (Ma et al., 2017). This research is focused on the characterization of the taxonomic position of the two strains.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and growth conditions

MS586\(^7\) and MS82 were isolated from a soybean rhizosphere sample by standard dilution plating on nutrient broth yeast extract (NBY) agar medium (Vidaver, 1967) at 28°C. Antimicrobial activity against multiple plant pathogens was detected with an antifungal plate assay as previously described (Gu, Wang, Chaney, Smith, & Lu, 2009). Following purification, the bacterium was preserved in 20% glycerol at −80°C. Pseudomonas spp. type strains and reference strains were provided by the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). All strains used in this study are summarized in Table A1.

2.2 | Cell morphology and physiological tests

Colony morphology of the strains MS586\(^7\) and MS82 was determined after growth on NBY agar plates. Gram staining was performed as described previously (Murray, Doetsch, & Robinow, 1994); cell morphology and flagellation types were observed with a transmission electron microscope (TEM) using routine negative glutaraldehyde staining; and the production of fluorescent pigments was tested on King B medium (King, Ward, & Raney, 1954). Optical density (OD600) metrics recorded for NBY liquid cultures were used to evaluate optimal growth and pH, at temperatures from 4°C to 40°C, with an interval of 4°C for 24 hr, and at pH 4.0–10.0.

Physiological and biochemical tests were conducted as described previously (Peix, Berge, Rivas, Abril, & Velázquez, 2005). Cellular fatty acids were identified using the Sherlock 6.1 system (Microbial IDentification Inc.) and the library RTSBA6 (Sasser, 1990). Biochemical features and enzyme activities were determined using API 20 NE and API 50 CH strips with API 50 CHB/E medium (bioMerieux), as well as Biology GENIII Microplates (Biolog) as directed in the manufacturer's instructions; results were recorded after incubation for 48 hr at 28°C.

2.3 | Phylogenetic analysis

Bacterial genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1987) and used as a template to amplify the nearly full-length 16S rRNA gene. PCR was performed with the 16S rRNA universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGACTTACCTTGTAGACT-3′) (Chelius & Triplet, 2000; Lane, 1991). Amplification and partial sequencing of rpoB (Tayeb, Ageron, Grimont, & Grimont, 2005), rpoD (Mulet, Bennasar, Lalucat, & García-Valdés, 2009), and gyrB (Yamamoto et al., 2000) housekeeping genes were performed following previously described methods (Mulet et al., 2009) using primers LAPS (5′-TGGCGGAGAACGAGCTCGGT-3′)/LAPS27 (5′-CAGCCTCTGTCACCCCGTT-3′) for rpoB, PsEG30F (5′-ATYGAATGCAATCGCA-3′)/PsEG790R (5′-CGTTGTATKT CCTTGA-3′) for rpoD, and APrU (5′-TGGAAGCCACGCGATTGCN GGRTCTTTYCYTGCRAG-3′)/UP1E (5′-CAGGAAACAGCTATGACC AYGNSNGGNAARTTYRA-3′) for gyrB. All PCR was performed with a PTC-200 Peltier Thermal Cycler (MJ Research), and products were purified using a Wizard SV Gel and PCR Clean-Up System (Promega). Sanger sequencing reactions were performed using the Eurofins MWG Operon.

Phylogenetic analysis of the multilocus sequence analysis (MLSA) was performed in MEGA 7 software using the maximum-likelihood algorithm (Kumar, Stecher, & Tamura, 2016). The sequence fragments of the four genes (16S rRNA, rpoB, rpoD, and gyrB) were concatenated in the following order: 16S rRNA, rpoB, rpoD, and gyrB. Sequences of type strains used in the MLSA were downloaded from
**TABLE 1** Differentiating characteristics of strain MS586<sup>T</sup> from other related species of *Pseudomonas*

| Characteristics         | 1 | 2<sup>a</sup> | 3<sup>b</sup> | 4<sup>c</sup> | 5<sup>c</sup> | 6<sup>d</sup> | 7<sup>e</sup> | 8<sup>e</sup> | 9<sup>e</sup> | 10<sup>e</sup> |
|-------------------------|---|---------------|---------------|---------------|---------------|--------------|--------------|--------------|-------------|--------------|
| Flagellation            | Polar, multiple | Polar, multiple | Polar, two | Polar, two | Polar, multiple | ND | ND | Polar, single | ND | ND |
| Fluorescence            | + | − | − | + | + | + | + | + | − | − |
| Growth at:              | 4°C | + | + | + | + | + | ND | ND | ND | ND |
| Tolerance of NaCl at    | 5% | + | − | + | + | − | + | − | − | − |
| Nitrate reduction       | − | − | − | − | − | − | − | + | + | − |
| Arginine dihydrolase    | + | + | + | + | + | + | − | + | − | + |
| Hydrolysis of gelatin   | + | − | + | − | − | + | − | − | − | − |
| Citrate utilization     | + | + | + | + | + | + | + | + | − | + |
| Urease                  | − | − | − | − | ND | − | − | − | − | − |
| Assimilation of         |    |    |    |    |    |    |    |    |    |    |
| L-Arabinose             | + | + | + | + | + | + | − | + | + | + |
| N-Acetyl-d-glucosamine  | + | + | + | + | + | + | + | + | − | − |
| Phenylacetic acid       | − | − | − | − | − | − | + | + | + | + |
| D-Mannose               | + | + | + | + | + | + | − | + | − | − |
| Dextrin                 | − | + | w | + | + | − | + | + | − | − |
| Tween-40                | − | + | + | + | + | + | − | + | + | − |
| D-Cellobiose            | − | + | − | + | + | − | + | + | − | − |
| D-Trehalose             | − | + | − | + | − | − | w | − | − | − |
| L-Arabinose             | + | + | + | + | + | + | − | + | + | + |
| D-Fructose              | + | + | + | + | + | + | ND | + | − | − |
| D-Mannitol              | + | + | + | + | + | + | + | + | − | + |
| D-Arabitol              | − | + | + | + | + | + | − | − | + | − |
| L-Alanine               | + | + | + | + | + | + | + | + | w | ND |
| L-Serine                | + | + | − | + | + | + | + | w | + | + |
| α-Ketobutyric acid      | − | − | w | + | + | − | + | − | + | − |
| α-Ketoglutaric acid     | + | + | + | + | − | + | + | + | − | + |
| Glucuronamide           | − | + | + | + | + | − | − | − | − | − |
| L-Histidine             | − | + | − | + | + | − | + | + | − | − |
| D-Serine                | − | + | w | + | + | − | + | − | − | − |
| D-Galactose             | + | + | + | + | + | − | + | + | − | + |
| D-Galacturonic acid      | − | ND | − | − | ND | − | − | + | − | + |
| D-Glucuronic acid       | − | − | − | − | − | − | − | − | − | + |
| Glucuronamide           | − | + | + | ND | + | − | − | − | − | ND |
| p-Hydroxy phenylacetic acid | − | − | − | − | − | − | − | + | − | − |
| Quinic acid             | + | + | + | + | + | + | − | + | + | + |
| D-Saccharic acid        | + | + | + | + | + | − | + | + | − | + |
| Glycyl-L-proline        | − | ND | + | + | + | − | + | + | − | + |
| L-Pyroglutamic acid     | + | + | + | + | ND | + | − | + | + | + |
| Inosine                 | − | + | + | + | + | + | − | + | − | − |
| Propionic acid          | + | + | + | + | + | + | w | − | − | − |
| Formic acid             | − | + | − | − | − | − | + | + | − | + |
| Acetic acid             | + | + | w | + | − | + | + | + | + | − |

(Continues)
NCBI (accession numbers in Table A2). The maximum-likelihood method was used to construct the phylogenetic tree with 1000 bootstrap replicates.

### 2.4 DNA fingerprinting

DNA fingerprinting has been evaluated and proposed as a reliable method for distinguishing different strains in the same taxon, which are not clonal varieties. Thus, the primer sequence corresponding to BOX elements (BoxA1R: 5′-CTACGGCAAGGCGACGCTGACG-3′) was used for DNA fingerprinting (Koeuth, Versalovic, & Lupski, 1995). PCR amplification was conducted as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles (94°C for 1 min, 52°C or 53°C for 1 min, and 72°C for 2 min), and finally 72°C for 8 min. The DNA fragments were analyzed in a 2% agarose gel.

### 2.5 Genome sequencing and analysis

Genomic DNA of strain MS586T was extracted using the Wizard Genomic DNA Purification Kit (Promega Corporation). The extracted genomic DNA was used for library construction with an average insert size of 400 bp, and three mate-pair libraries with an average insert size of 2000 bp, 5000 bp, and 8000 bp were prepared and sequenced on the Illumina MiSeq instrument according to the manufacturer’s instructions (Illumina). The standard library and 2000-bp mate-pair library were selected for de novo assembly using a method described by Durfee et al. (2008) using DNASTAR Lasergene software (DNASTAR, Inc.). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Angiuoli et al., 2008). The complete genome sequence was deposited in GenBank under accession number CP014205, and the genome project was deposited in the Genomes OnLine Database under GP0128017.

Similarity analyses (ANI and GGDC) of the sequenced genome of strain MS586T to other 40 genomes of the closely related Pseudomonas species were determined as briefed below. ANI based on pairwise comparison was calculated using the software JSpecies with the ANIb algorithm (Richter & Rosselló-Móra, 2009). GGDC was calculated using the web service http://ggdc.dsmz.de and using the recommended BLAST-method (Meier-Kolthoff et al., 2013). The GGDC results shown are based on the recommended formula 2 (sum of all identities found in HSPs divided by the overall HSP length), which is independent of the genome length and is thus robust against the use of incomplete draft genomes. The Type (Strain) Genome Server (https://www.dsmz.de/services/online-tools/tygs) with the recommended settings was used to clarify species delineation (Meier-Kolthoff & Göker, 2019). The phylogenomic tree based on whole-genome sequences was reconstructed by Genome Blast Distance Phylogeny (GBDP). Accession numbers of sequences used in the whole-genome phylogenetic analysis are summarized in Table A3. The clustering of the type-based species using a 70% dDDH radius around each type strain was conducted as previously described (Meier-Kolthoff & Göker, 2019).

### 2.6 Chemotaxonomic analysis

As important chemical characteristics for bacterial identification, the cellular fatty acid profile of the strain MS586T was analyzed. Cellular fatty acids were harvested after 2 days of growth at 28°C on TSA. Fatty acids extracted from the bacteria were methylated and analyzed following the protocol of the Sherlock 6.1 Microbial Identification (MIDI) system (Microbial IDentification Inc.) using the library RTSBA6 (Sasser, 1990). Analyses of respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ (Braunschweig, Germany).

### 3 RESULTS AND DISCUSSION

#### 3.1 Phenotype analysis

Both strains MS586T and MS82 were observed to be Gram-negative, rod-shaped (0.6–0.8 × 2.0–3.0 μm), and motile utilizing polar flagella (Figure A1). Colonies of the two strains were 3–5 mm in diameter and light yellow after 2 days of incubation on NBY at 28°C. No growth was detected at 40°C or with 7% NaCl. The optimum growth occurred at 28–30°C. The bacteria tolerated pH values ranging from 4 to 10. The two strains could produce fluorescent pigments when cultured for 24–48 hr at 28°C on King B medium, whereas Pseudomonas kribbensis 46-2T, which is the closest species of strains MS586T and MS82, could not produce fluorescent (Table 1). Strain MS586T showed negative for assimilation of dextrin, formic acid, glutamic acid, and Methyl pyruvate.
and ω-serine. In contrast, all these reactions were not negative for *P. kribbensis* 46-2<sup>T</sup>, *P. granadensis* F-278,770<sup>T</sup>, *P. moraviensis* 1B4<sup>T</sup>, and *Pseudomonas koreensis* Ps 9-14<sup>T</sup>. Gelatin was hydrolyzed by strain MS586<sup>T</sup>, but it was negative by *P. kribbensis* 46-2<sup>T</sup>. The physiological, morphological, and phenotypic characteristics in the API 20 NE, API 50 CH, and Biology GEN III tests, which allowed differentiation of strains MS586<sup>T</sup> from other closely related *Pseudomonas* species, are listed in Table 1.

### 3.2 | Phylogenetic analysis

Sequence analysis revealed that the 16S rRNA genes of MS586<sup>T</sup> and MS82 shared significant identities (>98%) to some *Pseudomonas* species of the *P. koreensis* subgroup in the *Pseudomonas fluorescens* group. The closely related strains include *P. kribbensis* 46-2<sup>T</sup> (99.94%), *P. granadensis* F-278,770<sup>T</sup> (99.55%), *P. koreensis* Ps 9-14<sup>T</sup> (99.52%), *P. reinekei* MT1<sup>T</sup> (99.46%), *P. moraviensis* 1B4<sup>T</sup> (99.41%), *P. vancouverensis* DhA-51<sup>T</sup> (99.33%), *P. baetica* a390<sup>T</sup> (99.20%), *P. jessenii* DSM 17150<sup>T</sup> (98.94%), and *P. fluorescens* Pf0-1 (99.87%). However, analysis of the 16S rRNA gene sequence alone is insufficient to define the relative taxonomic positions of *Pseudomonas* species (Rosselló-Móra & Whitman, 2018). Therefore, MLSA was conducted based on previously described methods using four gene sequences for the studies: 16S rRNA (1326 bp), *rpoB* (905 bp), *rpoD* (802 bp), and *gyrB* (663 bp). According to Hesse et al. (2018), the genus *Pseudomonas* has been phylogenetically divided into 13 groups (G) and 10 subgroups (SG). The closely related species of *P. fluorescens* subgroup and representative species of each group were selected to reconstruct the phylogenetic tree. The maximum-likelihood tree illustrates the phylogenetic position of strain MS586<sup>T</sup> and 61 related members of the genus *Pseudomonas* based on four concatenated gene sequences (3696 bp); *Acinetobacter baumannii* strain ATCC 19606<sup>T</sup> was used as an outgroup. As shown in Figure 1, strains MS586<sup>T</sup> and MS82 were clustered with *P. fluorescens* Pf0-1 with 100% bootstrap values. Strains MS586<sup>T</sup> and MS82 belong to the *P. koreensis* subgroup in the *P. fluorescens* group. It has been noted that, as reported by Gomila, Peña, Mulet, Lalucat, and García-Valdés (2015), 30% of the genus *Pseudomonas* sequenced genomes of non-type strains were not correctly assigned at the species level in the accepted taxonomy of the genus and 20% of the strains were not identified at the species level. Therefore, further extensive research is needed to update the *Pseudomonas* taxonomy.

### 3.3 | DNA fingerprinting

DNA fingerprinting by BOX-PCR revealed that strains MS586<sup>T</sup> and MS82 were different representatives of the proposed novel species. As shown in Figure A2, two strains have the two common bands (490 bp and 900 bp) in the BOX-PCR profiles; however, each of them produced unique bands (125 bp, 300 bp, 750 bp, and 1350 bp for MS586<sup>T</sup>; 700 bp, 750 bp, 1100 bp, and 1350 bp for MS82), which suggests the two strains are not identical isolates.

### 3.4 | General taxonomic genome features of strain MS586<sup>T</sup>

The main characteristics of the whole-genome sequence of strain MS586<sup>T</sup> are depicted in Table 2. No plasmid was detected. The DNA G+C content of strain MS586<sup>T</sup> was 60.48 mol%. This value is in the range (48–68 mol%) of those reported within the genus *Pseudomonas* (Hesse et al., 2018).

All genome-relatedness values of strain MS586<sup>T</sup> were calculated by the algorithms ANI and GGDC. The MS586<sup>T</sup> genome was compared with the complete genome assemblies downloaded from NCBI for the strains shown in Table 3. ANI > 95%–96% is equivalent to a DNA-DNA hybridization of 70% (Kim, Oh, Park, & Chun, 2014). The species demarcations ANI ≥ 95% or GGDC ≥ 70% were used as a benchmark (Richter & Rosselló-Móra, 2009). ANI values and GGDC values ranged from 75.28% to 98.24% and 21.00% to 84.10%, respectively, with the highest value between MS82 and MS586<sup>T</sup>. As shown in Table 3, strain MS586<sup>T</sup> shared less than 91% ANI and 35% GGDC with any of the other type strain of bacteria, but it had ANI value of 98.24% and GGDC value of 84.10% with strain MS82, which are higher than the species boundary cutoff values. Additionally, the two strains share 95.59% ANI and 65.30% GGDC with *P. fluorescens* Pf0-1, which is the closest relative outside to the novel species. As reported by Lopes et al. (Lopes et al., 2018), three strains isolated from tropical soils, which share ≥95% ANI values with strain MS586<sup>T</sup>, are the potential strains for the novel species. As shown in Figure 2, the whole-genome-based phylogenetic tree obtained with TYGS automated pipeline shows that both MS586<sup>T</sup> and MS82 were grouped into the same species cluster and confirmed that *P. kribbensis* 46-2<sup>T</sup> is the closely related type strain. *P. fluorescens* Pf0-1 was clustered to independent branch, which indicates its distinct phylogenetic position and potential as a separate species. Collectively, the ANI, GGDC, and whole-genome phylogenetic tree data support that strains MS586<sup>T</sup> and MS82 represent a unique species.

Furthermore, strains MS586<sup>T</sup> and MS82 were noteworthy, which were isolated from the rhizosphere of soybean plants associated with fungal pathogen infections. Strain MS586<sup>T</sup> has shown remarkable antifungal activities against a broad range of plant fungal pathogens (Jia and Lu, unpublished). Similarly, our study has demonstrated that strain MS82 possesses antifungal activities against the mushroom fungal pathogen *Mycogone perniciosa*, but not the mushroom fungus (Ma et al., 2019). Furthermore, it has been reported that *PafR* gene confers resistance to the mushroom pathogenic fungus (Ma et al., 2017). As expected, the *PafR* gene was also found in strains MS586<sup>T</sup>. Therefore, it is not surprising
that multiple nonribosomal peptide synthetase gene clusters, which are frequently associated with the production of antimicrobial compounds (Mootz & Marahiel, 1997), have been predicted from the genomes of the bacterial strains.

3.5 | Chemotaxonomic analysis

Cellular fatty acids were identified using the Sherlock 6.1 system (Microbial IDentification Inc.) and the library RTSBA6 (Sasser, 1990). The majority of fatty acids for strain MS586\textsuperscript{T} were C\textsubscript{16:0} (22.9%), summed feature 3 (C\textsubscript{16:1ω7c}/C\textsubscript{16:1ω6c}) (23.57%), summed feature 8 (C\textsubscript{18:1ω7c}/C\textsubscript{18:1ω6c}) (13.37%), and C\textsubscript{17:0 cyclo} (10.28%). The similarity of the fatty acid profiles supports the affiliation of strain MS586\textsuperscript{T} with the genus \textit{Pseudomonas}. The three fatty acids typical of the genus \textit{Pseudomonas} (C\textsubscript{10:0} 3-OH, C\textsubscript{12:0}, and C\textsubscript{12:0} 3-OH) were also identified in strain MS586\textsuperscript{T} (Palleroni, 2005). Besides, the lowest amounts of fatty acid C\textsubscript{16:0} (22.9%) were observed in strain MS586\textsuperscript{T} than in the strains of closely related species (29.4–36.5%). Strain MS586\textsuperscript{T} also contains the highest amounts of C\textsubscript{16:0} 3-OH (6.6%) when compared to the reference strains (2.2%–5.4%). The detailed fatty acid profiles of strain MS586\textsuperscript{T} and the type strains of closely related species are provided in Table 4. Two-dimensional TLC analysis revealed that the polar lipid of strain MS586\textsuperscript{T} were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidyglycerol (PG), three unidentified phospholipids (PL), and one unidentified lipid (L) (Figure A3). Strain MS586\textsuperscript{T} contains higher amounts of PL and L as compared with those of the closest relative of \textit{P. kribbensis} 46-2\textsuperscript{T}. As expected, the major polar lipid components of strain MS586\textsuperscript{T} were PE, DPG, and PG, which agrees with data published previously for the genus \textit{Pseudomonas} (Moore et al., 2006). Also, the major respiratory quinone of strain MS586\textsuperscript{T} was Q-9, which is consistent with other species in the genus \textit{Pseudomonas} (Moore et al., 2006).

4 | CONCLUSIONS

Analyses of molecular, phenotypic, physiological, and biochemical characteristics are needed to discriminate between members of the genus \textit{Pseudomonas} and other rRNA groups of aerobic ‘pseudomonads’ (Palleroni, 2005). These analyses of strains MS586\textsuperscript{T} and MS82 revealed its distinct characteristics of 16S rRNA and housekeeping gene sequences, ANI values, GGDC values, and phenotypic and chemotaxonomic assays as compared with those of other species and strains of the genus \textit{Pseudomonas}. Collectively, these results demonstrate that strain MS586\textsuperscript{T} and strain MS82 represent a novel species of the genus \textit{Pseudomonas}. The name \textit{Pseudomonas glycinae} sp. nov. is proposed with strain MS586\textsuperscript{T} as the type strain. Strain MS586\textsuperscript{T} is a motile Gram-negative, rod-shaped, strictly aerobic, catalase- and oxidase-positive, fluorescent strain. These findings support the placement of strain MS586\textsuperscript{T} in the genus \textit{Pseudomonas} (Hildebrand, Palleroni, Hendson, Toth, & Johnson, 1994).

### TABLE 2 Chromosome statistics for strain MS586\textsuperscript{T}

| Feature   | Total |
|-----------|-------|
| Size      | 6,396,728 bp |
| Genes     | 5893 |
| CDs       | 5805 |
| Pseudogenes | 131 |
| rRNAs     | 17 |
| tRNAs     | 67 |
| ncRNA     | 4 |
| G+C content | 60.48% |

4.1 | Description of \textit{Pseudomonas glycinae} sp. nov.

\textit{Pseudomonas glycinae} (gly.c'i'nae. N.L. gen. n. glycinae of Glycine max, soybean) is an aerobic, Gram-negative, rod-shaped bacterium, with motility through polar flagella. When cultured on NBY agar plates, it produces fluorescence and forms fresh light-yellow colonies. The colony is raised from the side view, the shape is circular, and it is usually 3.0–5.0 mm in diameter within 2 days of growth at 28°C. Cells are 0.6–0.8 μm. Growth occurs between 4°C and 36°C (optimum growth temperature is 28–30°C). Growth occurs between pH 4 and 10 (optimum pH 6–7). The organism tolerates up to 6% (w/v) NaCl. The results obtained with Biology GENIII Microplates indicate the following substrates can be utilized: α-d-glucose, d-mannose, d-fructose, d-fucose, d-galactose, d-mannitol, l-alanine, l-arginine, l-aspartic acid, l-glutamic acid, l-pyroglutamic acid, l-serine, d-glucuronic acid, mucic acid, quinic acid, d-saccharic acid, l-lactic acid, citric acid, α-ketoglutaric acid, l-malic acid, γ-amino butyric acid, β-hydroxy-d-,l-butyric acid, propionic acid, acetic acid, and N-acetyl-d-glucosamine, but negative for dextrin, d-maltose, d-trehalose, d-cellobiose, gentiobiose, sucrose, stachyose, d-raffinose, α-d-lactose, d-melibiose, β-methyl-d-glucoside, d-salicin, N-acetyl-β-d-mannosamine, N-acetyl-d-galactosamine, N-acetyl neuraminic acid, 3-methyl glucose, l-rhamnose, inosine, d-sorbitol, d-arabitol, myo-inositol, d-glucose-6-PO\textsubscript{4}, d-fructose-6-PO\textsubscript{4}, d-aspartic acid, d-serine, gelatin, glycy-l-proline, l-histidine, pectin, d-galacturonic acid, l-galactonic acid lactone, d-glucuronic acid, glucuronamide, p-hydroxy-phenylacetic acid, methyl pyruvate, d-lactic acid methyl ester, d-malic acid, Tween-40,
α-hydroxybutyric acid, α-ketobutyric acid, acetoacetic acid, and formic acid. According to API 20 NE tests, the organism is positive for the hydrolysis of gelatin, arginine dihidrolase, and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, and trisodium citrate, but negative for the reduction of nitrate to nitrogen and nitrogen, indole production, glucose fermentation, urease, hydrolysis of esculin and β-galactosidase, and assimilation of maltose, adipic acid,
and phenylacetic acid. According to API 50 CH tests, the organism is positive for acid production from L-arabinose, D-ribose, D-xylene, D-mannose, D-mannitol, and D-fucose, but negative for erythritol, D-arabinose, L-xylene, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, D-fructose, D-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose,
d-rafﬁnose, starch, glycogen, xylitol, gentiobiose, d-turanose, d-lyxose, d-tagatose, l-fucose, d-arabitol, l-arabitol, potassium 2-ke
togluconate, and potassium 5-ke togluconate. The predominant quinone system is Q9. Polar lipids are diphasphatidylglycerol, phosp hatidylethanolamine, phosphatidylglycerol, three unidenti
cied phospholipids, and one unidentified lipid. The type strain is MS586\(^7\) (LMG 30275\(^1\), NRRL B-65441\(^1\)), isolated from the rhizosphere of soybean grown in Mississippi. The DNA G+C content of the type strain is 60.48 mol%.  

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CONFLICT OF INTEREST  
None declared.

AUTHOR CONTRIBUTIONS  
Jiayuan Jia: Formal analysis (equal); visualization (equal); writing – original draft (equal). Xiaoxiang Wang: Formal analysis (equal); investigation (equal); writing – original draft (equal). Peng Deng: Formal analysis (equal). Lin Ma: Formal analysis (equal); resources (equal). Sonya M. Baird: Methodology (equal). Xiangdong Li: Formal analysis (equal); funding acquisition (equal). Shi-En Lu: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); project administration (equal); writing – original draft (equal); writing – review & editing (equal).

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

The GenBank accession numbers for the complete genome of Pseudomonas glycinae MS586\(^7\) and the full-length sequence of 16S rDNA are CP014205 and MG692779, respectively. The type strain MS586\(^7\) was deposited in the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA (Culture collection 1 accession #NRRL B-6544: https://nrnlncaur.usda.gov/cgi-bin/usda/prokaryote/report.html?nrrlcodes=B-65441), and the BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium (Culture collection 2 accession #LMG 30275; https://bccm.belspo.be/catalogues/lmg-strain-details?NUM=30275).

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REFERENCES

Angiuoli, S. V., Gussman, A., Klimke, W., Cochrane, G., Field, D., Garrity, G. M., ... White, O. (2008). Toward an online repository of standard operating procedures (SOPs) for (meta) genomic annotation. OMICS A Journal of Integrative Biology, 12(2), 137-141. https://doi.org/10.1089/omi.2008.0017

Camara, B., Strömpl, C., Verbag, S., Špršer, C., Pieper, D. H., & Tindall, B. J. (2007). Pseudomonas reinekei sp. nov., Pseudomonas moorei sp. nov. and Pseudomonas mohnii sp. nov., novel species capable of degrading chlorosaliclylates or isomiparinic acid. International Journal of Systematic and Evolutionary Microbiology, 57(5), 923–931. https://doi.org/10.1099/ijss.0.04703-0

Chang, D.-H., Rhee, M.-S., Kim, J.-S., Lee, Y., Park, M. Y., Kim, H., ... Kim, B.-C. (2016). Pseudomonas kribbensis sp. nov., isolated from garden soils in Daejeon, Korea. Antonie Van Leeuwenhoek, 109(1), 1433–1446. https://doi.org/10.1007/s10482-016-0743-0

Chelius, M. K., & Tripplett, E. W. (2000). Immunocalization of dinitro
genese reductase produced by Klebsiella pneumoniae in association with Zea mays L. Applied and Environmental Microbiology, 66, 783–787. https://doi.org/10.1128/AEM.66.2.783-787.2000

Doyle, J. J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11–15.

Durfee, T., Nelson, R., Baldwin, S., Plunkett, G., Burland, V., Mau, B., ... Blattner, F. R. (2008). The complete genome sequence of Escherichia coli DH10B: Insights into the biology of a laboratory workhorse. Journal of Bacteriology, 190(7), 2597–2606. https://doi.org/10.1128/JB.01695-07

Furmanczyk, E. M., Kaminski, M. A., Lipinski, L., Dziembowski, A., & Sobczak, A. (2018). Pseudomonas laurysulfatovorans sp. nov., sodium dodecyl sulfate degrading bacteria, isolated from the peaty soil of a wastewater treatment plant. Systematic and Applied Microbiology, 41(4), 348–354. https://doi.org/10.1016/j.syapm.2018.03.009

Gomila, M., Peña, A., Mulet, M., Lalucat, J., & García-Valdés, E. (2015). Phylogenomics and systematics in Pseudomonas. Frontiers in Microbiology, 6, 214. https://doi.org/10.3389/fmicb.2015.00214

Gu, G., Wang, N., Chaney, N., Smith, L., & Lu, S.-E. (2009). AmbR1 is a key transcriptional regulator for production of antifungal activity of Burkholderia contaminans strain MS514. FEMS Microbiology Letters, 297(1), 54–60. https://doi.org/10.1111/j.1574-6968.2009.01653.x

Hesse, C., Schulz, F., Bull, C. T., Shaffer, B. T., Yan, Q., Shapiro, N., ... Paulsen, I. T. (2018). Genome-based evolutionary history of Pseudomonas spp. Environmental Microbiology, 20(6), 2142–2159. https://doi.org/10.1111/1462-2920.14130

Hildebrand, D., Palleroni, N., Henderson, M., Toth, J., & Johnson, J. (1994). Pseudomonas ﬂavescens sp. nov., isolated from walnut blight cankers. International Journal of Systematic and Evolutionary Microbiology, 44, 410–415. https://doi.org/10.1099/00207713-4-3-410

Kim, M., Oh, H.-S., Park, S.-C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. International Journal of Systematic and Evolutionary Microbiology, 64, 346–351. https://doi.org/10.1099/ijss.0.059774-0

King, E. O., Ward, M. K., & Raney, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. The Journal of Laboratory and Clinical Medicine, 44(2), 301–307.

Koethe, T., Versalovic, J., & Lupschi, J. R. (1995). Differential subsequence conservation of interspersed repetitive Streptococcus pneumonae BOX elements in diverse bacteria. Genome Research, 5(4), 408–418. https://doi.org/10.10110/gr.5.4.408

Konstantinidis, K. T., & Tiedje, J. M. (2005). Genomic insights that ad
vance the species definition for prokaryotes. Proceedings of the National Academy of Sciences of the United States of America, 102(7), 2567–2572. https://doi.org/10.1073/pnas.0409727102

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolution genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7), 1870-1874. https://doi.org/10.1093/molbev/msw054
APPENDIX A

FIGURE A1  The cellular morphology of strain MS586\textsuperscript{T} was observed by transmission electron microscopy

FIGURE A2  Fingerprinting analysis of strain MS586\textsuperscript{T} and strain MS82 based on analysis of BOX-PCR: 1, strain MS586\textsuperscript{T}; 2, strain MS82; Mk: 1-kb DNA ladder (GoldBio) was used with markers

FIGURE A3  Two-dimensional TLC of polar lipids of strain MS586\textsuperscript{T}. DPG, diphosphatidylglycerol; L, lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid

TABLE A1  List of strains used in this study

| Species                  | Strain  | Source collection |
|--------------------------|---------|-------------------|
| *Pseudomonas glycinae*   | MS586   | This study        |
| *Pseudomonas glycinae*   | MS82    | Ma et al. (2017)  |
| *Pseudomonas moraviensis*| 1B4     | DSMZ              |
| *Pseudomonas jessenii*   | CIP105274 | DSMZ           |
| *Pseudomonas reinekei*   | MT1     | DSMZ              |
| *Pseudomonas vancouverensis* | DhA-51 | DSMZ            |
| *Pseudomonas baetica*    | a390    | DSMZ              |

Note: DSMZ: German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.
| Species                | Gene name | Accession number | Strain designation | Species                | Gene name | Accession number | Strain designation |
|------------------------|-----------|------------------|--------------------|------------------------|-----------|------------------|--------------------|
| *P. glycinea*          | rpoB      | CP014205         | MS586\(^T\)        | *P. glycinea*          | rpoB      | CP028826         | MS82               |
| *P. glycinea*          | rpoD      | CP014205         | MS586\(^T\)        | *P. glycinea*          | rpoD      | CP028826         | MS82               |
| *P. glycinea*          | gyrB      | CP014205         | MS586\(^T\)        | *P. aeruginosa*        | rpoB      | CP012001         | DSM 50071\(^T\)    |
| *P. fluorescens*       | rpoB      | CP000094         | P0-1               | *P. fluorescens*       | rpoB      | CP012001         | DSM 50071\(^T\)    |
| *P. fluorescens*       | rpoD      | CP000094         | P0-1               | *P. fluorescens*       | rpoD      | CP012001         | DSM 50071\(^T\)    |
| *P. fluorescens*       | gyrB      | CP000094         | P0-1               | *P. fluorescens*       | gyrB      | CP012001         | DSM 50071\(^T\)    |
| *P. anguilliseptica*   | rpoB      | FNSC000000000    | DSM 12111\(^T\)    | *P. arseneoxydans*     | rpoB      | LT629705          | CECT 7543\(^T\)    |
| *P. avellanae*         | rpoB      | AKB500000000     | BPIC 631\(^T\)     | *P. baetica*           | rpoB      | PKLC000000000    | a390\(^T\)         |
| *P. bolearica*         | rpoB      | CP007511         | DSM 6083\(^T\)     | *P. bauzanensis*       | rpoB      | FOG000000000     | DSM 22558\(^T\)    |
| *P. brassicacearum*    | rpoB      | LT629713         | LMG 21623\(^T\)    | *P. brenneri*          | rpoB      | VFIL000000000    | DSM 15294\(^T\)    |
| *P. capeferrum*        | rpoB      | JM100000000      | WCS358\(^T\)       | *P. corrugata*         | rpoB      | LHVK000000000    | DSM 7228\(^T\)     |
| *P. cauliflora*        | rpoB      | BBIQ000000000    | NBRC 16637\(^T\)   | *P. gessardii*         | rpoB      | VFEW000000000    | DSM 17152\(^T\)    |
| *P. graminis*          | rpoB      | FOHW000000000    | DSM 11363\(^T\)    | *P. granadensis*       | rpoB      | LT629778          | LMG 27940\(^T\)    |
| *P. helmantiensis*     | rpoB      | HG405037         | OHA11\(^T\)        | *P. jessenii*          | rpoB      | NIWT010000000    | DSM 17150\(^T\)    |

(Continues)
| Species              | Gene name | Accession number | Strain designation | Species              | Gene name | Accession number | Strain designation |
|----------------------|-----------|------------------|--------------------|----------------------|-----------|------------------|--------------------|
| P. koreensis         | 16S rRNA  | LT629687         | LMG 21318<sup>T</sup> | P. kribbensis        | 16S rRNA  | CP029608         | 46-2<sup>T</sup>   |
|                      | rpoB      | LT629687         | LMG 21318<sup>T</sup> |                      | rpoB      | CP029608         | 46-2<sup>T</sup>   |
|                      | rpoD      | LT629687         | LMG 21318<sup>T</sup> |                      | rpoD      | CP029608         | 46-2<sup>T</sup>   |
|                      | gyrB      | LT629687         | LMG 21318<sup>T</sup> |                      | gyrB      | CP029608         | 46-2<sup>T</sup>   |
| P. laurylsulfatiphila| 16S rRNA  | LT629687         | LMG 21318<sup>T</sup> | P. laurylsulfativorans | 16S rRNA  | MUJK000000000    | AP3_22<sup>T</sup> |
|                      | rpoB      | LT629687         | LMG 21318<sup>T</sup> |                      | rpoB      | MUJK000000000    | AP3_22<sup>T</sup> |
|                      | rpoD      | LT629687         | LMG 21318<sup>T</sup> |                      | rpoD      | MUJK000000000    | AP3_22<sup>T</sup> |
|                      | gyrB      | LT629687         | LMG 21318<sup>T</sup> |                      | gyrB      | MUJK000000000    | AP3_22<sup>T</sup> |
| P. licanensis        | 16S rRNA  | JYLH000000000    | DSM 17149<sup>T</sup> | P. lini              | 16S rRNA  | JYLB000000000    | DSM 16768<sup>T</sup> |
|                      | rpoB      | JYLH000000000    | DSM 17149<sup>T</sup> |                      | rpoB      | JYLB000000000    | DSM 16768<sup>T</sup> |
|                      | rpoD      | JYLH000000000    | DSM 17149<sup>T</sup> |                      | rpoD      | JYLB000000000    | DSM 16768<sup>T</sup> |
|                      | gyrB      | JYLH000000000    | DSM 17149<sup>T</sup> |                      | gyrB      | JYLB000000000    | DSM 16768<sup>T</sup> |
| P. linyingensis      | 16S rRNA  | FNZE000000000    | LMG 25967<sup>T</sup> | P. litoralis         | 16S rRNA  | LT629748         | 2SM5<sup>T</sup>   |
|                      | rpoB      | FNZE000000000    | LMG 25967<sup>T</sup> |                      | rpoB      | LT629748         | 2SM5<sup>T</sup>   |
|                      | rpoD      | FNZE000000000    | LMG 25967<sup>T</sup> |                      | rpoD      | LT629748         | 2SM5<sup>T</sup>   |
|                      | gyrB      | FNZE000000000    | LMG 25967<sup>T</sup> |                      | gyrB      | LT629748         | 2SM5<sup>T</sup>   |
| P. lundensis         | 16S rRNA  | JYKY000000000    | DSM 6252<sup>T</sup>  | P. lutea             | 16S rRNA  | JRMBO000000000   | DSM 17257<sup>T</sup> |
|                      | rpoB      | JYKY000000000    | DSM 6252<sup>T</sup>  |                      | rpoB      | JRMBO000000000   | DSM 17257<sup>T</sup> |
|                      | rpoD      | JYKY000000000    | DSM 6252<sup>T</sup>  |                      | rpoD      | JRMBO000000000   | DSM 17257<sup>T</sup> |
|                      | gyrB      | JYKY000000000    | DSM 6252<sup>T</sup>  |                      | gyrB      | JRMBO000000000   | DSM 17257<sup>T</sup> |
| P. mandelii          | 16S rRNA  | LT629796         | LMG 21607<sup>T</sup> | P. migulae           | 16S rRNA  | FNTY000000000    | LMG 21608<sup>T</sup> |
|                      | rpoB      | LT629796         | LMG 21607<sup>T</sup> |                      | rpoB      | FNTY000000000    | LMG 21608<sup>T</sup> |
|                      | rpoD      | LT629796         | LMG 21607<sup>T</sup> |                      | rpoD      | FNTY000000000    | LMG 21608<sup>T</sup> |
|                      | gyrB      | LT629796         | LMG 21607<sup>T</sup> |                      | gyrB      | FNTY000000000    | LMG 21608<sup>T</sup> |
| P. mohnii            | 16S rRNA  | FNRV010000000    | DSM 18327<sup>T</sup> | P. moorei            | 16S rRNA  | VZPP000000000    | CCUG 53114<sup>T</sup> |
|                      | rpoB      | FNRV010000000    | DSM 18327<sup>T</sup> |                      | rpoB      | VZPP000000000    | CCUG 53114<sup>T</sup> |
|                      | rpoD      | FNRV010000000    | DSM 18327<sup>T</sup> |                      | rpoD      | VZPP000000000    | CCUG 53114<sup>T</sup> |
|                      | gyrB      | FNRV010000000    | DSM 18327<sup>T</sup> |                      | gyrB      | VZPP000000000    | CCUG 53114<sup>T</sup> |
| P. moraviensis       | 16S rRNA  | LT629788         | LMG 24280<sup>T</sup> | P. oleovorans        | 16S rRNA  | UGU000000000    | NCTC10692<sup>T</sup> |
|                      | rpoB      | LT629788         | LMG 24280<sup>T</sup> |                      | rpoB      | UGU000000000    | NCTC10692<sup>T</sup> |
|                      | rpoD      | LT629788         | LMG 24280<sup>T</sup> |                      | rpoD      | UGU000000000    | NCTC10692<sup>T</sup> |
|                      | gyrB      | LT629788         | LMG 24280<sup>T</sup> |                      | gyrB      | UGU000000000    | NCTC10692<sup>T</sup> |
| P. oryzihabitans     | 16S rRNA  | BBIT000000000    | NBRC 102199<sup>T</sup> | P. otitidis          | 16S rRNA  | FOJP000000000    | DSM 17224<sup>T</sup> |
|                      | rpoB      | BBIT000000000    | NBRC 102199<sup>T</sup> |                      | rpoB      | FOJP000000000    | DSM 17224<sup>T</sup> |
|                      | rpoD      | BBIT000000000    | NBRC 102199<sup>T</sup> |                      | rpoD      | FOJP000000000    | DSM 17224<sup>T</sup> |
|                      | gyrB      | BBIT000000000    | NBRC 102199<sup>T</sup> |                      | gyrB      | FOJP000000000    | DSM 17224<sup>T</sup> |

(Continues)
| Species                  | Gene name | Accession number | Strain designation | Species                  | Gene name | Accession number | Strain designation |
|--------------------------|-----------|------------------|--------------------|--------------------------|-----------|------------------|--------------------|
| *P. panipatensis*        | 16S rRNA  | FNDS0000000000   | CCM 7469<sup>T</sup> | *P. peli*                | 16S rRNA  | FMTL0000000000   | DSM 17833<sup>T</sup> |
|                          | rpoB      | FNDS0000000000   | CCM 7469<sup>T</sup> |                          | rpoB      | FMTL0000000000   | DSM 17833<sup>T</sup> |
|                          | rpoD      | FNDS0000000000   | CCM 7469<sup>T</sup> |                          | rpoD      | FMTL0000000000   | DSM 17833<sup>T</sup> |
|                          | gyrB      | FNDS0000000000   | CCM 7469<sup>T</sup> |                          | gyrB      | FMTL0000000000   | DSM 17833<sup>T</sup> |
| *P. pertucinogena*       | 16S rRNA  | AB021380         | IFO 14163<sup>T</sup> | *P. proseki*             | 16S rRNA  | LT629762         | LMG 26867<sup>T</sup> |
|                          | rpoB      | AJJ17441         | LMG 1874<sup>T</sup>  |                          | rpoB      | LT629762         | LMG 26867<sup>T</sup> |
|                          | rpoD      | FN554502         | LMG 1874<sup>T</sup>  |                          | rpoD      | LT629762         | LMG 26867<sup>T</sup> |
|                          | gyrB      | DQ350613         | JCM 11950<sup>T</sup> |                          | gyrB      | LT629762         | LMG 26867<sup>T</sup> |
| *P. psychrotolerans*     | 16S rRNA  | FMWB0000000000   | DSM 15758<sup>T</sup> | *P. punonensis*          | 16S rRNA  | FRBQ0000000000   | CECT 8089<sup>T</sup> |
|                          | rpoB      | FMWB0000000000   | DSM 15758<sup>T</sup> |                          | rpoB      | FRBQ0000000000   | CECT 8089<sup>T</sup> |
|                          | rpoD      | FMWB0000000000   | DSM 15758<sup>T</sup> |                          | rpoD      | FRBQ0000000000   | CECT 8089<sup>T</sup> |
|                          | gyrB      | FMWB0000000000   | DSM 15758<sup>T</sup> |                          | gyrB      | FRBQ0000000000   | CECT 8089<sup>T</sup> |
| *P. putida*              | 16S rRNA  | AP013070         | NBRC 14164<sup>T</sup> | *P. reinekei*            | 16S rRNA  | MSTQ0000000000   | MTI<sup>T</sup> |
|                          | rpoB      | AP013070         | NBRC 14164<sup>T</sup> |                          | rpoB      | MSTQ0000000000   | MTI<sup>T</sup> |
|                          | rpoD      | AP013070         | NBRC 14164<sup>T</sup> |                          | rpoD      | MSTQ0000000000   | MTI<sup>T</sup> |
|                          | gyrB      | AP013070         | NBRC 14164<sup>T</sup> |                          | gyrB      | MSTQ0000000000   | MTI<sup>T</sup> |
| *P. resinovorans*        | 16S rRNA  | AUJE0000000000   | DSM 21078<sup>T</sup> | *P. sagittaria*          | 16S rRNA  | FOXM0000000000   | JCM 18195<sup>T</sup> |
|                          | rpoB      | AUJE0000000000   | DSM 21078<sup>T</sup> |                          | rpoB      | FOXM0000000000   | JCM 18195<sup>T</sup> |
|                          | rpoD      | AUJE0000000000   | DSM 21078<sup>T</sup> |                          | rpoD      | FOXM0000000000   | JCM 18195<sup>T</sup> |
|                          | gyrB      | AUJE0000000000   | DSM 21078<sup>T</sup> |                          | gyrB      | FOXM0000000000   | JCM 18195<sup>T</sup> |
| *P. straminea*           | 16S rRNA  | FOM0010000000    | JCM 2783<sup>T</sup>  | *P. stutzeri*            | 16S rRNA  | CP002881         | CGMCC 1.1603<sup>T</sup> |
|                          | rpoB      | FOM0010000000    | JCM 2783<sup>T</sup>  |                          | rpoB      | CP002881         | CGMCC 1.1603<sup>T</sup> |
|                          | rpoD      | FOM0010000000    | JCM 2783<sup>T</sup>  |                          | rpoD      | CP002881         | CGMCC 1.1603<sup>T</sup> |
|                          | gyrB      | FOM0010000000    | JCM 2783<sup>T</sup>  |                          | gyrB      | CP002881         | CGMCC 1.1603<sup>T</sup> |
| *P. synxantha*           | 16S rRNA  | LR590482         | NCTC10696<sup>T</sup> | *P. syringae*            | 16S rRNA  | JALK0000000000   | DSM 10604<sup>T</sup> |
|                          | rpoB      | LR590482         | NCTC10696<sup>T</sup> |                          | rpoB      | JALK0000000000   | DSM 10604<sup>T</sup> |
|                          | rpoD      | LR590482         | NCTC10696<sup>T</sup> |                          | rpoD      | JALK0000000000   | DSM 10604<sup>T</sup> |
|                          | gyrB      | LR590482         | NCTC10696<sup>T</sup> |                          | gyrB      | JALK0000000000   | DSM 10604<sup>T</sup> |
| *P. taeanensis*          | 16S rRNA  | AW5Q0000000000   | MS-3<sup>T</sup>      | *P. taetrolea*           | 16S rRNA  | LS483370         | NCTC 10697<sup>T</sup> |
|                          | rpoB      | AW5Q0000000000   | MS-3<sup>T</sup>      |                          | rpoB      | LS483370         | NCTC 10697<sup>T</sup> |
|                          | rpoD      | AW5Q0000000000   | MS-3<sup>T</sup>      |                          | rpoD      | LS483370         | NCTC 10697<sup>T</sup> |
|                          | gyrB      | AW5Q0000000000   | MS-3<sup>T</sup>      |                          | gyrB      | LS483370         | NCTC 10697<sup>T</sup> |
| *P. tolaasii*            | 16S rRNA  | PHHD0000000000   | NCPPB 2192<sup>T</sup> | *P. toyotomiensis*       | 16S rRNA  | NIQV0000000000   | DSM 26169<sup>T</sup> |
|                          | rpoB      | PHHD0000000000   | NCPPB 2192<sup>T</sup> |                          | rpoB      | NIQV0000000000   | DSM 26169<sup>T</sup> |
|                          | rpoD      | PHHD0000000000   | NCPPB 2192<sup>T</sup> |                          | rpoD      | NIQV0000000000   | DSM 26169<sup>T</sup> |
|                          | gyrB      | PHHD0000000000   | NCPPB 2192<sup>T</sup> |                          | gyrB      | NIQV0000000000   | DSM 26169<sup>T</sup> |
### Table A2 (Continued)

| Species          | Gene name | Accession number | Strain designation | Species          | Gene name | Accession number | Strain designation |
|------------------|-----------|------------------|--------------------|------------------|-----------|------------------|--------------------|
| *P. tremae*      | 16S rRNA  | LJRO0000000000   | ICMP9151<sup>T</sup> | *P. umsongensis* | 16S rRNA  | NIWU0000000000  | DSM 16611<sup>T</sup> |
|                  | rpoB      | LJRO0000000000   | ICMP9151<sup>T</sup> |                  | rpoB      | NIWU0000000000  | DSM 16611<sup>T</sup> |
|                  | rpoD      | LJRO0000000000   | ICMP9151<sup>T</sup> |                  | rpoD      | NIWU0000000000  | DSM 16611<sup>T</sup> |
|                  | gyrB      | LJRO0000000000   | ICMP9151<sup>T</sup> |                  | gyrB      | NIWU0000000000  | DSM 16611<sup>T</sup> |
| *P. vancouverensis* | 16S rRNA | RRZK0000000000   | Dha-51<sup>T</sup>  | *Acinetobacter baumannii* | 16S rRNA | MJHA0000000000  | ATCC 19606<sup>T</sup> |
|                  | rpoB      | RRZK0000000000   | Dha-51<sup>T</sup>  |                  | rpoB      | MJHA0000000000  | ATCC 19606<sup>T</sup> |
|                  | rpoD      | RRZK0000000000   | Dha-51<sup>T</sup>  |                  | rpoD      | MJHA0000000000  | ATCC 19606<sup>T</sup> |
|                  | gyrB      | RRZK0000000000   | Dha-51<sup>T</sup>  |                  | gyrB      | MJHA0000000000  | ATCC 19606<sup>T</sup> |

### Table A3

Accession numbers of the sequences of different *Pseudomonas* spp. strains used in the whole-genome phylogenetic analysis

| Species               | Accession number | Strain designation |
|-----------------------|------------------|--------------------|
| *P. glycinae*         | GCA_001594225    | MS586<sup>T</sup>  |
| *P. glycinae*         | GCA_003055645    | MS82               |
| *P. fluorescens*      | GCA_000012445    | PFO-1              |
| *P. arsenicoxydans*   | GCA_900103875    | CECT 7543<sup>T</sup> |
| *P. baetica*          | GCA_002813455    | LMG 25716<sup>T</sup> |
| *P. batumici*         | GCA_000820515    | UCM B-321<sup>T</sup> |
| *P. chlororaphis*     | GCA_001269625    | LMG 5004<sup>T</sup> |
| *P. frederiksbergensis* | GCA_900105495 | LMG 19851<sup>T</sup> |
| *P. granadensis*      | GCA_900105485    | LMG 27940<sup>T</sup> |
| *P. jessenii*         | GCA_002236115    | DSM 17150<sup>T</sup> |
| *P. koreensis*        | GCA_900101415    | LMG 21318<sup>T</sup> |
| *P. kribbensis*       | GCA_003352185    | 46-2<sup>T</sup>   |
| *P. laurylsulfatiphila* | GCA_002934665  | AP3_16<sup>T</sup> |
| *P. laurylsulfativorans* | GCA_002906155 | AP3_22<sup>T</sup> |
| *P. lini*             | GCA_001042905    | DSM 16768<sup>T</sup> |
| *P. moorei*           | GCA_900102045    | DSM 12647<sup>T</sup> |
| *P. moraviensis*      | GCA_900105805    | LMG 24280<sup>T</sup> |
| *P. prosekii*         | GCA_900105155    | LMG 26867<sup>T</sup> |
| *P. reinekei*         | GCA_001945365    | MT1<sup>T</sup>    |
| *P. vancouverensis*   | GCA_900105825    | LMG 202221<sup>T</sup> |