Taxonomic Description template

Pseudomonas campi sp. nov., a nitrate-reducing bacterium isolated from grassland soil

1.1 Author names

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1.4 Keyword

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1.5 Repositories:

The NCBI accession numbers for the genome and 16S rRNA gene sequences of *Pseudomonas campi* sp. nov. S1-A32-2^T are CP053697 and MT415401, respectively

ABSTRACT

A new strain was isolated from grassland soil that has the potential to assimilate ammonium by the reduction of nitrate in the presence of oxygen. Whole genome sequence analysis shows the presence of an assimilatory cytoplasmic nitrate reductase gene *nasA* and the assimilatory nitrite reductase genes *nirBD* which are involved in the sequential reduction of nitrate to nitrite and further to ammonium, respectively. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolate belongs to the genus *Pseudomonas*. The closest phylogenetic neighbours based on 16S rRNA gene sequence analysis are the type strains of *Pseudomonas peli* (98.15%) and *Pseudomonas guineae* (97.88%). Computation of the average nucleotide index (ANI) and digital DNA-DNA hybridization (dDDH) with closest phylogenetic neighbours of S1-A32-2^T revealed genetic differences at the species level, which were further substantiated by differences in several physiological characteristics. Based on these results, it was concluded that the soil isolate represents a novel species of the genus *Pseudomonas,* for which the name *Pseudomonas campi* sp. nov. (type strain S1-A32-2^T = LMG 31521^T = DSM 110222^T) is proposed.

The genus *Pseudomonas*, described by Migula in 1894, is one of the most complex genera of the *Gammaproteobacteria* with a large number of species that have been continuously subjected to taxonomical studies over the recent decades [1-4]. Members of the genus *Pseudomonas* can be isolated from a wide variety of habitats such as soils, water, sewage, plants, animals, humans etc. [5]. Colonization of various ecological niches is attributed to their metabolic, physiologic and genomic versatility [6]. It has also been observed that different functions of nitrogen (N) transformation processes in the N cycle can be found in this genus, for instance the uptake of nitrate (NO_3^-) by *P. fluorescens* for assimilation purposes [7] or the anaerobic NO_3^- respiration by *Pseudomonas aeruginosa* [8], i.e. a canonical reduction of NO_3^- to dinitrogen (N_2) with nitrite (NO_2^-), gaseous nitric (NO) and nitrous oxide (N_2O) as intermediates in a well-known dissimilatory process called denitrification. On the other hand, *Pseudomonas alcaliphila* [9] and *Pseudomonas putrefaciens* [10] are involved in NO_3^- ammonification. This is considered as a crucial N transformation process that retains N in terrestrial ecosystems in the less mobile form ammonium (NH_4^+) and makes it available to primary producers, whereas gaseous reduction products through denitrification contribute substantially to N losses in soils [11, 12].

With the motive to investigate the biodiversity of NO_3^-/NO_2^- reducing bacteria, a high-throughput cultivation experiment was performed on the research platform Biodiversity Exploratories located in Germany (www.biodiversity-exploratories.de). To investigate the impact of land use intensity on this bacterial group, soil samples were taken from differently managed grassland plots. Several selective media and enrichment cultures have been used, which differ in N source, C/N ratio, pH and carbon source to cover a wide range of diversity in the bacterial isolates. A first taxonomic classification was performed by a similarity analysis using MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry) combined with a 16S rRNA gene analysis. In this context, a NO_3^- reducing strain with the designation S1-A32-2^T was isolated, which could not be assigned to a hitherto known species. Therefore, in this study the exact taxonomic position of this strain was investigated by a polyphasic approach. Furthermore, physiological characteristics and the gene inventory in relation to the N-transformation processes were studied to determine the isolate's potential to participate in N transformation processes.

Isolation and Ecology

Strain S1-A32-2^T was isolated from the top soil (peat soil) of a grassland plot of the exploratory Schorfheide-Chorin (Germany, State Brandenburg 53° 5' 14.7" N; 13° 58' 10.7" E). The study plot was located on a meadow that was extensively managed in the years prior to sampling. It was usually cut twice a year and not fertilized. In the year before sampling, additional grazing with sheep was carried out. The preparation of soil samples and isolation of strains was performed according to a study conducted previously [13]. The strain was isolated from medium G3M12 described by Heylen *et al.* [14] which is characterized by a C/N ratio of 2.5, pH 7.5, nitrate as mineral N source and ethanol as a carbon source. The strain was purified by streak dilution on nutrient agar II (NAII; SIFIN, Germany) and culturing at 22 °C for 3- 5 days, and then stored at -80°C in nutrient broth II (NBII; SIFIN, Germany) containing 40% glycerol until further processing.

16S RNA phylogeny

For estimation of the taxonomic affiliation, the nearly complete 16S rRNA gene of S1-A32-2^T was sequenced as described previously [15]. The pairwise 16S rRNA gene sequence comparison reveals *Pseudomonas peli* (98.15%) as the closest phylogenetic neighbour (https://www.ezbiocloud.net [16]). For all other *Pseudomonas* species, values below 98.0% were determined (Tab. 1). Considering the species boundary of 98.2-99.0% for the 16S rRNA sequence similarity which was derived from a comparative study between DNA-DNA hybridization and 16S rRNA gene analysis by Meier-Kolthoff *et al.* [17], or the value of 98.65% corresponding to the average nucleotide identity (ANI) threshold determined by Kim *et al.* [18], a separate species position of the investigated strain is indicated.

For a more detailed investigation of the phylogenetic position within the genus *Pseudomonas*, the type strains of closely related species as well as representative strains from subgroups categorized for the genus by García-Valdés and Lalucat [3] were selected. A sequence alignment of 1333 nt was used as basis for the calculation. The evolutionary history of the strain was inferred using Maximum-likelihood (ML) as well as Neighbour-joining (NJ) algorithms based on general time reversible model and maximum composite likelihood, respectively. Trees were calculated using the discrete gamma distributions and evolutionary invariable model (G+I) with bootstrap values based on 100 resampling using Mega7 software (Molecular Evolutionary Genetic Analysis, version 7.0; [19]). Phylogenetic trees derived from the analysis of 16S rRNA gene sequences show that strain S1-A32-2^T forms a cluster together with *P. anguilliseptica, P. peli,* and *P. guineae*, which belong to the *P. anguilliseptica* subgroup of the *Pseudomonas fluorescens* lineage, by both treeing methods (Fig.1)**.** The clustering of strain S1-A32-2^T with *P. anguilliseptica*, *P. peli* and *P. guineae* was supported by a bootstrap value of 82%.

The analysis of housekeeping genes is very helpful to obtain higher resolution at the intrageneric level than with 16S rRNA genes alone within *Pseudomonas* [2, 3]. The *rpoD* gene (encoding the sigma70 subunit of the RNA polymerase gene) in particular has a high discriminatory power at species level. Therefore, phylogenetic trees were constructed based on amino acid based alignments of in silico translated *rpoD* genes with a length of 627 AA using MEGA 7 software. Trees were obtained using ML and NJ methods based on the WAG and Poisson models. In contrast to the 16S rRNA gene based phylogeny, strain S1-A32-2^T formed a cluster with *Pseudomonas alcaligenes* and *Pseudomonas aeruginosa*

which indicates an affiliation to the *P. aeruginosa* lineage of the genus (Fig. S1, supplemental material). However, this position is only supported by a bootstrap value of 33 %.

Genome Features

To support the results of the phylogenetic analysis and to get deeper taxonomic insights, the genome of S1-A32-2^T was sequenced and analysed. Genome sequencing was carried out on the PacBio Sequel Platform (Pacific Biosciences, Menlo Park, CA) using Sequel Binding Kit 3.0, Sequencing Plate 3.0, and SMRT cell 1M v2 (PacBio). Briefly, DNA from cells grown in liquid nutrient broth medium at 25°C for 24h was extracted using the Genomic-tip 20/G kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. Genomic DNA was sheared to approximately 10 kb using g-TUBEs (Covaris Inc., Woburn, MA) and further processed according to the protocol. Library preparation was performed applying the SMRTbell template prep kit 1.0 SPv3 and Barcoded overhang adapter kit- 8 A according to the Procedure & Checklist – Preparing Multiplexed Microbial SMRTbell® Libraries for the PacBio® Sequel® System (PacBio). The prepared SMRTBell® libraries were loaded according to the diffusion loading protocol (PacBio). The multiplexed library including S1-A32-2^T was loaded on one SMRT cells in the concentration of 5 pM. Movie time was 10 h per SMRT cell after immobilization for 2 h and pre-extension for 2 h. After data demultiplexing, genome assembly for S1-A32-2^T was performed using the Microbial Assembly pipeline as embedded in SMRT Link version 8.0.0.80529 (PacBio), utilizing 262,061 realigned subreads, with an average subread length of 4,193 bp. As a result, the complete genome sequence of 4,436,925 bp was obtained forming one circular contig. The sequence was annotated using the rapid annotation using subsystem technology, version 2.0 (RAST) [20, 21]. The genome statistics of strain S1-A32-2^T is provided in Table 2.

To confirm the novel species position of strain S1-A32-2^T as revealed by 16S rRNA gene comparisons, overall genome relatedness indices [(OGRI); 22] were calculated to analyse the relationship with closely related species (Tab. 1). ANI values were calculated by the OrthoANIu procedure [23]. Digital DNA–DNA hybridization (dDDH) and genomic G+C content via the genome to genome distance calculator [(GGDC); 17]. The OGRI values obtained (Tab. 1) were below the species thresholds of 95–96% for ANI [18, 24, 25] and 70% for dDDH [17] and therefore support the affiliation of strain S1-A32-2^T to a novel species. The G+C content of 63.43% determined for S1-A32-2^T was within the genus range and similar to the G+C content calculated for the closest phylogenetically related species (62.97 – 66.52%) as shown in the Table 1. It lies well in the range of the G+C values of the genus *Pseudomonas* (58–69 mol%) [26]. Moreover, the differences calculated between the DNA G+C content of S1-A32-2^T and related type strains are greater than 1% (Tab. 1) further substantiating affiliation to a distinct species [27]. Thus, ANI values coupled with dDDH similarity values and DNA G+C content data corroborate that S1-A32-2^T represents a novel species within the genus of *Pseudomonas*.

Physiology and Chemotaxonomy

In addition to the demarcation on the genetic level, a polyphasic approach also requires a differentiation of a novel species on the phenotypic level. Accordingly, fatty acid methyl esters were determined for S1-A32-2^T and 16S rRNA gene analysis based closely related type strains (Fig. 1), namely, *P. peli* DSM 17833^T, *P. guineae* DSM 21282^T and *P. anguilliseptica* DSM 12111^T. The analysis of cellular fatty acid was performed by the German Collection of Microorganisms and Cell Cultures GmbH (DSZM). The

strains were cultured on nutrient broth medium at 22°C for 3 days and the extraction and preparations were conducted according to the standard protocol of the Sherlock Microbial Identification system (MIDI, version 6.1). Analysis and identification of fatty acids were carried out using a Hewlett Packard HP 6890 gas chromatograph and TSBA database version 4.10. The predominant cellular fatty acids found in S1-A32-2^T were $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{16:0}$. Minor amounts were found for $C_{12:0}$, $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$, $C_{15:0}$, and $C_{14:0}$. The profile was similar to that of the related species (Table S1, supplemental material). However, some quantitative differences could be found between the most abundant fatty acids, while qualitative differences were found only for fatty acids that were present in minor amounts or traces.

Furthermore, MALD-TOF MS was performed to differentiate S1-A32-2^T from the closely related strains. Prior to measurement, bacterial strains were inoculated on NAII and cultivated for 24 h at 25°C. The whole-cell measurement protocol was used to obtain mass spectra [28]. The MS analysis was performed with a Microflex™ LT/SH MALDI-TOF mass spectrometer (Bruker Daltonics) using Flex Control 3.4 software, described in detail by Müller *et al.* [29]. As shown in Fig. 2, strain S1-A32-2^T shows a high spectral dissimilarity from its closest phylogenetic neighbours which clearly supports the separate species position.

Morphological and physiological tests, such as the motility of cells, oxidase and catalase activities were performed as described by Behrendt *et al.* [30]. Gram-reaction was determined by the rapid KOH string test [31]. Furthermore, the GEN III microplates (Biolog) and the API 20NE test system (bioMérieux) were applied according to the manufacturer's instructions. The reactions in the test panels were read after 48 h of incubation at 25°C. Results for strain S1-A32-2^T are shown in Table 3 and the species description. It can be clearly distinguished from related species due to several differentiating physiological characteristics. For instance, amino acid L-histidine could solely be utilized by the investigated strain. Furthermore, it was tested negative for bromo-succinic acid, lithium chloride, D- and L-serine which were found positive for its closest phylogenetic neighbours. Further characteristics with discriminating power are shown in Table 3.

These results also provide phenotypic support for the separate species status that was shown by the phylogenetic analysis. Therefore, the assignment of the soil isolate to a novel species, *Pseudomonas campi* sp. nov., is proposed.

Genome Annotation and Evaluation of the mineral N transformation pathway

The gene inventory of strain S1-A32-2^T was searched for genes with relation to N transformation. An assimilatory nitrate reductase could be identified at position 2323983-2326724 (CP053697) starting with the alternative start codon GTG. BLAST searches confirmed this annotation which could be differentiated from respiratory (membrane-bound) and dissimilatory (periplasmic) nitrate reductases. This enzyme of S1-A32-2^T showed a similarity of more than 77% across the total length of the 913 AA to the other cytoplasmic assimilatory nitrate reductases (NasA) of the genus *Pseudomonas*. Further, the nitrite reductase NirBD could be found in the genome (CP053697: 2971029-2973584; CP053697: 2973584-2973961). This protein is known as an assimilatory nitrite reductase but is also involved in the fermentative pathway of dissimilatory nitrate reduction to ammonium [11]. With reference to this, Sun *et al.* [32] demonstrated the formation of ammonium by this enzyme.

For the experimental investigation of nitrate reduction strain S1-A32-2^T was grown in mineral medium [33] containing 5 mM KNO_3 or KNO_2. Serum vials (120 ml) were filled with 50 ml medium and sealed with butyl rubber stoppers. For anoxic tests, the head space was flushed three times with helium and an overpressure was established. A pre-culture incubated in mineral medium containing NO_3^- or NO_2^- was used as inoculum (1% $v_{preculture}/v_{fresh\ medium}$) to start the growth monitoring. The growth experiments were conducted three times. The controls were replicated twice to check for abiotic N_2O or NH_4^+ production. Headspace and liquid samples (1 ml each) were taken at the start of incubation and every 24 h for 7 days. Headspace samples were transferred to 3 ml helium filled Exetainer (Labco Limited, UK) and measured via GC [Agilent 7890, Agilent Technologies Inc., USA; N_2 and O_2 with a thermal conductivity detector, CO_2 and N_2O with a pulsed-discharge helium ionization detector]. 100 µl of liquid samples were used for growth measurement by optical density (OD_{600}) with a microplate reader (Tecan Infinite M Plex, Switzerland) corrected for 1 cm path length. The remaining liquid samples were centrifuged for 5 min at 12000*g* and the supernatants were frozen at -20°C until further use. NH_4^+, NO_3^- and NO_2^- were determined colorimetrically [34]. The strain was not able to anaerobically reduce NO_3^- and NO_2^-. However, under aerobic conditions NO_3^- and NO_2^- were consumed but no NH_4^+ was accumulated in the medium. Small amounts of N_2O were formed, which most likely originated from the detoxification of NO_2^- under oxic conditions. These results indicate that the strain used the mineral nitrogen species NO_3^- and NO_2^- for assimilation, and that the predicted NH_4^+ formed is not released into the medium, but is completely utilized for cell metabolism.

Protologue

Description of *Pseudomonas campi* sp. nov.

Pseudomonas campi (cam'pi. L. gen. n. *campi* of a field, of grassland).

Cells are Gram-stain-negative, aerobic, motile and non-spore forming in nature. Colonies grown on nutrient agar medium as well as on trypticase soya agar medium are round and whitely translucent after 3-4 days of incubation at 25°C. No fluorescent pigment is formed. Cell growth is observed at 37°C, but not at 4°C. Optimum temperature for growth is 25°C. Sodium chloride is tolerated between 0 to 3% (w/v), with no growth observed at 4% or 8%. The strain reduces nitrate and nitrite. Positive for oxidase and catalase. In the GEN III test system L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-malic acid, tween 40, α-hydroxy-butyric acid, propionic acid, acetic acid and formic acid is oxidized. Negative reaction is shown for dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachysoe, Draffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-insitol, glycerol, D-glucose-6-PO4, D-fructose-6-PO4, D-aspartic acid, D- and L-serine, glycl-L-proline, L-pyroglutamic acid, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, bromo-succinic acid, γ-amino-butyric acid, α-keto-butyric acid. Weakly positive results are observed for: gelatine, glucuronamide, methyl pyruvate, β-hydorxy-D, L-butyric acid and acetoacetic acid. Resistance is shown to toroleandomycin, rifamycin SV, lincomycin, guanidine HCl (weak), niaproof 4, vancomycin, potassium tellurite and aztreonam (weak), whereas it is vulnerable for fusidic acid, D-serine, minocycline, nalidixic acid, lithium chloride, sodium butyrate and sodium bromate. In the API 20NE test, indole

production, glucose fermentation, arginine dihydrolase, urease, β-glucosidase, protease and β-galactosidase is negative. Assimilation of malic acid is positive whereas negative results are obtained for D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Major fatty acids include $C_{18:1}\omega7c$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, and $C_{16:0}$, while minor components are $C_{12:0}$, $C_{10:0}$ 3-OH, $C_{12:0}$ 3-OH, $C_{17:1}\omega8c$, $C_{15:0}$, and $C_{14:0}$.

The type strain S1-A32-2^T (LMG = 15321^T = DSM 110222^T) was isolated from the top soil of an extensively managed grassland plot in the Schorfheide-Chorin exploratory of the long-term biodiversity research platform in Germany (53° 5′ 14.7″ N; 13° 58′ 10.7″ E). The DNA G+C content of the type strain is 63.43%.

Genome sequence accession number: CP053697.

16S rRNA gene accession number: MT415401.

AUTHOR STATEMENTS

1.6 Authors and contributors

Conceptualization: T, UB, AU, StK, MAH; Formal Analysis: T, UB, AU, TS, BUF, SuK; Writing – original draft: T; Writing – review & editing: UB, BUF, StK, TS, MAH, AU.

1.7 Conflicts of interest

The authors declare that there are no conflicts of interest.

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1.9 Ethical approval

Not required.

1.10 Consent for publication

Not required.

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ABBREVIATIONS

AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; TYGS, Type (Strain) Genome Server; OGRI, overall genome relatedness indices.

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FIGURES AND TABLES

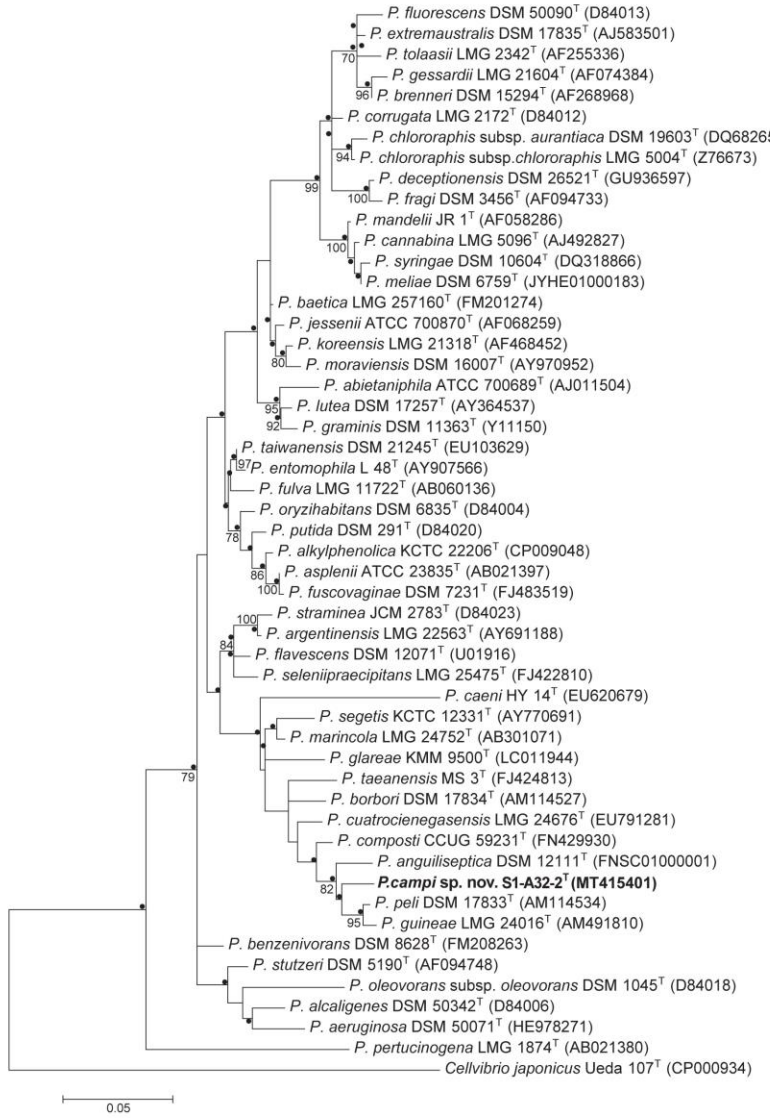

Figure 1. Maximum–likelihood tree on the basis of 16S rRNA gene sequences showing the phylogenetic position of the strain S1-A32-2^T among type strains of the genus *Pseudomonas*. Filled circles indicate branches of the tree that were also obtained using the neighbour-joining method. Numbers at branch nodes refer to bootstrap values >70%. *Cellvibrio japonicus* Ueda107^T was used as the outgroup. Bar, 0.05 substitutions per nucleotide site.

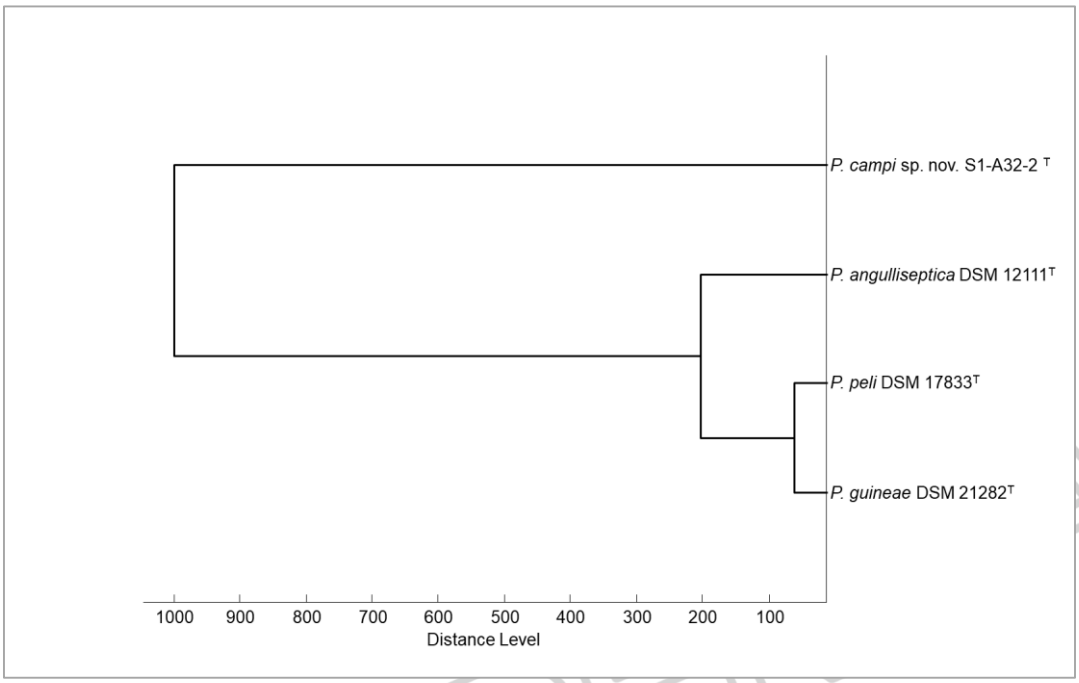

Figure 2. Cluster anaylsis showing the similarities of the MALDI-TOF mass spectra of strain S1-A32-2^T and type strains of closely related *Pseudomonas* species. Obtained profiles were used to generate a dendogram using BioTyper (version 3.1, Brucker Daltonics).

Table 1. dDDH values, G+C content, ANI and16S rRNA gene similarity values calculated for strain S1-A32-2^T and for genomes of closely related species.

Strains	dDDH values (%)	G+C content (%)	G+C content difference (%)	ANI (%)	Similarity 16S rRNA gene (%)
P. campi sp. nov. (S1-A32-2^T)	-	63.43	-	-	-
P. composti (CCUG 59231^T)	22.90	62.97	1.47	79.52	97.88
P. guineae (LMG 24016^T)	21.70	58.88	4.55	78.44	97.88
P. peli (DSM 17833^T)	23.10	60.15	3.28	79.26	98.15
P. aeruginosa (DSM 50071^T)	22.50	66.52	3.09	78.96	97.03
P. alcaligenes (NBRC 14167^T)	26.90	65.49	2.06	83.58	97.19
P. anguilliseptica (DSM 12111^T)	24.40	58.90	4.53	78.71	97.06
P. cuatrocienegasensis (CIP 109853T)	23.00	62.02	1.41	78.06	97.55

Table 2. General statistics of *Pseudomonas campi* sp. nov. S1-A32-2^T genome.

Genome	*Pseudomonas campi* sp. nov. S1-A32-2^T
Size (bp)	4,436,925
G+C content (%)	63.5
L50	1
Number of contigs (with PEGs)	1
Number of subsystems	316
Number of coding sequences	4036
Number of RNAs	67

406 **Table 3.** Physiological characteristics that differentiate strain S1-A32-2^T from its closest phylogenetic
407 relatives.

408 Strains: 1, *Pseudomonas campi* sp. nov. S1-A32-2^T; 2, *Pseudomonas guineae* DSM 21282^T; 3, *Pseudo-*
409 *monas anguilliseptica* DSM 12111^T; and *Pseudomonas peli* DSM 17833^T. +, Positive; -, negative; w,
410 weak reaction. Data are from this study.

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Characteristic	**1**	**2**	**3**	**4**
Assimilation of (GEN III)				
L-Malic acid	+	+	+	-
L -Aspartic Acid	+	+	-	-
α-Hydroxybutyric acid	+	-	-	+
L -Histidine	+	-	-	-
Formic acid	+	w	-	+
Bromosuccinic acid	-	+	+	+
D-Malic acid	-	+	+	w
α-Ketoglutaric acid	-	+	-	+
Citric acid	-	+	-	+
L-Lactic acid	-	+	-	+
D-Raffinose	-	-	+	-
D-Turanose	-	-	w	-
Sodium bromate	-	-	w	-
Sodium butyrate	-	-	w	-
3-Methyl glucose	-	-	-	w
D- and L-Serine	-	w	+	+
α-Hydroxybutyric acid	w	+	-	+
Methyl pyruvate	w	+	-	+
Acetoacetic acid	w	-	-	+
Gelatin	w	-	-	w
Glucuroamide	w	w	w	+
Tolerance to following				
Lithium chloride	-	+	w	+
Nalidixic acid	-	+	w	-
Fusidic Acid	-	-	w	-
Minocycline	-	-	w	-
Aztreonam	w	+	w	+
Guanidine HCL	w	-	w	+
4% Sodium chloride	-	+	w	+
8% Sodium chloride	-	w	w	+

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Online supplementary material:

Pseudomonas campi sp. nov., a nitrate-reducing bacterium isolated from grassland soil

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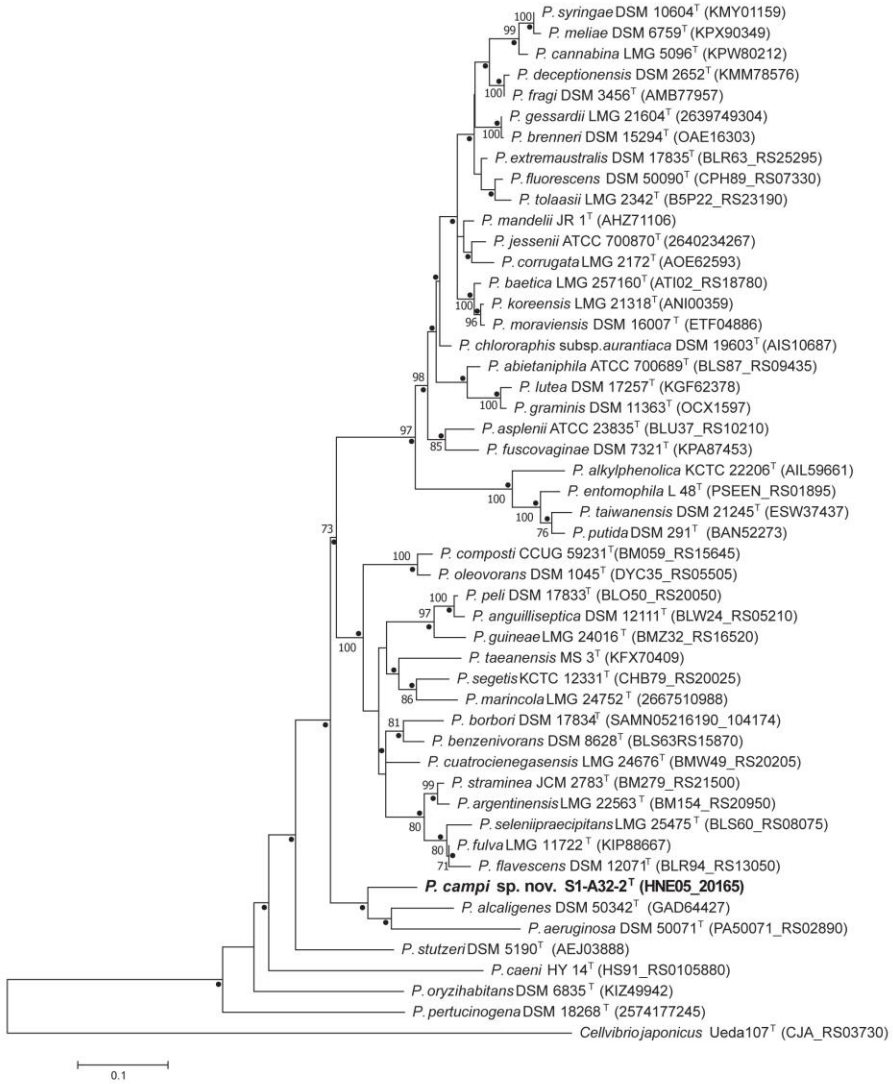

Figure S1. Maximum-likelihood tree based on RpoD shows phylogenetic position of the strain S1-A32-2^T among type strains of the genus *Pseudomonas*. Filled circles indicate branches of the tree that were also obtained using the neighbour-joining method. Trees were generated using 100 bootstrap replications on the basis on the amino acid alignment. Numbers at branch nodes refers to bootstrap values (>70%). *Cellvibrio japonicus* Ueda107^T was used as the out-group. Bar, 0.10 substitutions per nucleotide site.

Table S1. Cellular fatty acid compositions of S1-A32-2^T and related species.

Strains: 1, *Pseudomonas campi* sp. nov. S1-A32-2^T; 2, *Pseudomonas guineae* DSM 21282^T; 3, *Pseudomonas anguilliseptica* DSM 12111^T; and *Pseudomonas peli* DSM 17833^T. TR, <1%; −, undetected. Data are from this study. Summed feature 1 and 3 contains iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH and $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, respectively.

Fatty acid	1	2	3	4
$C_{9:0}$ 3-OH	TR	TR	-	TR
$C_{10:0}$	TR	TR	TR	TR
$C_{10:0}$ 3-OH	5,37	3,52	2,77	4,03
$C_{11:0}$	TR	TR	TR	TR
$C_{11:0}$ 3-OH	TR	TR	-	TR
iso-$C_{11:0}$ 3-OH	TR	TR	TR	TR
$C_{12:0}$	5,62	5,28	3,98	6,07
$C_{12:0}$ 2-OH	TR	TR	TR	TR
$C_{12:0}$ 3-OH	4,25	3,89	3,05	4,91
$C_{12:1}$ 3-OH	TR		TR	-
$C_{13:0}$	TR	TR	-	TR
iso-$C_{13:0}$	TR	TR	-	TR
$C_{14:0}$	1,26	TR	TR	TR
$C_{14:1}\omega5c$	TR	-	-	TR
$C_{15:0}$	1,97	2,16	1,1	5,42
iso-$C_{15:0}$	-	-	-	TR
$C_{15:1}\omega6c$	TR	TR	-	1,88
$C_{15:1}\omega8c$	TR	TR	-	TR
$C_{16:0}$	12,57	14,27	23,03	9,18
$C_{16:0}$ 3-OH	TR	-	-	-
iso-$C_{16:0}$	-	TR	TR	-
$C_{16:1}\omega5c$	-	TR	-	-
$C_{17:0}$	1,08	1,49	TR	TR
iso-$C_{17:0}$	TR	TR	TR	TR
$C_{17:1}\omega6c$	TR	TR	TR	1,52
$C_{17:1}\omega8c$	2,01	1,45	TR	3,73
$C_{18:0}$	TR	TR	TR	TR
$C_{18:1}\omega5c$	-	TR	-	-
$C_{18:1}\omega7c$	34,43	24,97	31,57	24,6
$C_{18:1}\omega7c$ 11-methyl	-	TR	-	-
$C_{19:0}$ 10-methyl	TR	-	-	-
$C_{20:1}\omega7c$	TR	TR	-	-
$C_{19:1}$ $\omega6c$	-	TR	-	TR
Summed feature 1	TR	TR	-	TR
Summed feature 3	20,52	33,75	27,71	24,43
unknown (ECL 11.799)	4,55	3,38	2,93	4,74