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Pseudomonas pharmafabricae sp. nov., Isolated From Pharmaceutical Wastewater

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Abstract

A Gram-stain-negative, aerobic, rod-shaped bacterial strain, designated ZYSR67-Z^T, was isolated from a pharmaceutical wastewater sample collected from a chemical factory in Zhejiang, China. The strain was motile by a single polar flagellum and grew at 4–42 °C (optimum, 35 °C), pH 5.0–9.0 (optimum, 6.0) and 0–5.0% (w/v) NaCl (optimum, 2.0%). Based on multilocus sequence analysis using 16S rRNA, *gyrB, rpoB* and *rpoD*, the strain ZYSR67-Z^T formed a distinct phylogenetic group in the genus *Pseudomonas*. The average nucleotide identity values between strain ZYSR67-Z^T and the closely related 10 type strains of the *Pseudomonas* species were 75.8–78.6%. The in silico DNA–DNA hybridization values indicated that strain ZYSR67-Z^T and the type strains of the *Pseudomonas* shared 21.4–23.1% DNA relatedness. The predominant isoprenoid quinone system was ubiquinone-9 while ubiquinone-8 was present in trace amounts. The major fatty acids (> 10%) identified were $C_{12:0}$, $C_{16:0}$, $C_{18:1}$ $\omega 7c$ and summed features 3 ($C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH). The major polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The genomic DNA G+C content was 62.6 mol%. On the basis of morphological, physiological and chemotaxonomic characteristics, together with the results of phylogenetic analysis, strain ZYSR67-Z^T was proposed to represent a novel species of the genus *Pseudomonas*, named *Pseudomonas pharmafabricae* sp. nov.. The type strain is ZYSR67-Z^T (=CGMCC 1.15498^T=JCM 31306^T).

Introduction

The genus *Pseudomonas*, belonging to the family *Pseudomonadaceae* was first described by Migula [16]. At the time of writing, this genus comprises 232 validly named species (http://www.bacterio.net/pseudomonas.html). This genus is typically featured as being Gram-staining-negative, non-spore forming, rod-shaped, having one or more polar flagella, and with Q-9 as the major respiratory quinone. Most members of this genus are aerobic, but some are (facultatively) anaerobic using NO_3 as alternate electron acceptor. The DNA G+C content of this genus is generally 58–69 mol% [10, 18, 29, 37].

Pseudomonas is one of the most diverse and ubiquitous bacterial genera whose species have been isolated from a variety of environments, such as soils, plants, animals, mineral waters, marine environments and clinical materials [20]. Members of this genus display great importance in wastewater treatment processes due to their high biodegradation capability of many organic compounds [33]. A few bacteria belonging to the 'viable but non-culturable' (VBNC) state have already been isolated from soil and wastewater through adding the resuscitation-promoting factor (Rpf) produced by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene, *gyr*B, *rpo*B and *rpo*D sequences of strain ZYSR67-Z^T are KX910087, KY887561, KY887563 and KY887562, respectively. WGS whole-genome project accession number for strain ZYSR67-Z^T is PIYS00000000. The Digital Protologue database Taxon Number for strain ZYSR67-Z^T is TA00200.

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Micrococcus luteus JCM 21373^T [7, 35]. Some were subsequently classified as belonging to novel species [12, 24, 35]. In this study, using the same strategy, we describe a recovered VBNC bacteria strain ZYSR67-Z^T isolated from a pharmaceutical wastewater sample. Based on the polyphasic taxonomic analysis, the isolate represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas pharmafabricae* sp. nov. is proposed.

Materials and Methods

Strains and Culture Conditions

Strain ZYSR67-Z^T was isolated from a pharmaceutical wastewater sample collected in Jinhua, Zhejiang province, China (29°12′N 119°64′E). Enrichment and isolation of this strain were performed as described by Yu et al. [35]. Purified strain ZYSR67-Z^T was routinely cultured on LB medium (Tryptone 10 g, Yeast extract 5 g, NaCl 10 g, H_2O 1000 mL, pH 7.0) at 35 °C and was preserved as suspensions with 20% (v/v) glycerol at − 80 °C. Two type species, *Pseudomonas guguanensis* JCM 18416^T [14] and *P. alcaligenes* JCM 20561^T [6], were selected as references for physiological and chemotaxonomic analyses. Both of them were obtained from the Japan Collection of Microorganisms (JCM) and cultured under the same conditions as strain ZYSR67-Z^T.

Phenotypic Characterization

Light microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) were used to observe the morphological characteristics of the strain cells cultured in the log-growth phase [30]. Motility was determined by inoculation in 0.5% Semi-solid agar LB agar medium and then observed whether colonies were radial or out diffusion. Optimal growth conditions and growth range were tested on LB medium. The temperature range for growth was tested at 4–45 °C (4, 10, 12, 15, 20, 28, 30, 35, 37, 40, 42 and 45 °C). pH range for growth was measured from 4.5 to 10.0 (0.5 unit intervals) by supplementing 40 mM buffering agents, including MES (pH 4.5–6.0), PIPES (pH 6.5–7.5), tricine (pH 8.0–8.5) and CAPSO (pH 9.0–10.0). Tolerance of NaCl (0–10.0%, w/v, at intervals of 1%) was investigated using NaCl-free LB medium. Anaerobic growth was examined in an anaerobic jar (MGC) with AnaeroPack (MGC) on modified LB agar supplemented with sodium thiosulphate (20 mM), sodium sulphite (5 mM), sodium sulphate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM) and L-arginine (5.0 g L^{-1}) as potential electron acceptors for 15 days.

Gram-staining reaction was carried out according to Claus [1]. Catalase activity was determined by utilizing 3% (v/v) H_2O_2 and oxidase activity was determined by oxidase reagent (bioMérieux). Hydrolysis of starch, gelatin, casein and tyrosine was tested according to Dong and Cai [3]. H_2S production test was assayed on the basis of Zhang et al. [36]. Hydrolysis of Tweens 20, 40, 60 and 80 was examined as described by Sun et al. [25]. The other physiological and biochemical characteristics were tested using API ZYM, API 20NE, API 50CH strips (BioMérieux) and the GN2 MicroPlate (Biolog Inc., USA) according to the manufacturer's instructions, which were observed after incubation at 35 °C for 4, 48, 48 h and 7 days, respectively. Antibiotic susceptibility test was carried out by antibiotics discs (WS/T125-1999) on LB medium plate, including ampicillin (10 µg), tobramycin (10 µg), nystatin (100 µg), rifamycin (5 µg), erythromycin (15 µg), bacitracin (0.04 U), ofloxacin (5 µg), vancomycin (30 µg), kanamycin (30 µg), clindamycin (2 µg), doxycycline (30 µg) and ciprofloxacin (5 µg).

Phylogenetic Analysis of 16S rRNA, Housekeeping Genes and Whole Genome

The genomic DNA of strain ZYSR67-Z^T was extracted using a Quick Bacteria Genomic DNA Extraction kit (DongSheng Biotech). The 16S rRNA gene sequence was amplified using general primer pair 8F (5′-AGAGTTTGATCCTGG CTCAG-3′) and 1510R (5′-GGCTACCTTGTTACGACT T-3′) [2], the PCR products were cloned into pClone007 Vector (Tsingke) and the positive clones were selected and sequenced. The resulting almost-complete 16S rRNA gene sequence (1462 bp) was subjected to pairwise sequence alignment by the EzTaxon server 2.1 (http://www.ezbiocloud.net/; Yoon et al. [34]). To further ascertain the phylogenetic position of the strain ZYSR67-Z^T, three housekeeping genes *gyrB, rpoB* and *rpoD* [14, 31] were amplified using primers UP-1 (5′-CAYGCNGGNGGNAARTTYGA-3′) and UP-2r (5′-CCRTCNACRTCNGCRTCNGTCAT-3′) (*gyrB*), LAPS (5′-TGGCCGAGA ACCAGTTCCGCGT-3′) and LAPS27 (5′-CGGCTTCGTCCAGCTTGTTCAG-3′) (*rpoB*), and 70F (5′-ACGACTGACCCGGTACGCATGTAYATGMGN-GARATGGGNACNGT-3′) and 70R (5′-ATAGAAATA ACCAGACGTAAGTTNGCYTCNACCATYTCYTTY TT-3′) (*rpoD*). The *gyrB, rpoB* and *rpoD* gene sequences of ZYSR67-Z^T were compared with their phylogenetically related sequences using the Blast program of the GenBank database. A pairwise sequence alignment was achieved using the CLUSTAL_X program, version 1.8 [28]. Phylogenetic trees were constructed by the methods of neighbour-joining [22], maximum-parsimony [5] and maximum-likelihood [26] with the software package MEGA 6.0 [27], and evaluated by bootstrap analysis [4] after 1000 replications.

The genome of strain ZYSR67-Z^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Novogene Technology Company). The sequencing

generated approximately 1.43 Gbyte of clean data (approximate 250-fold genome coverage). The de novo assembly of the reads was performed using ABySS 2.0.2 [23]. The assembly *k*-value was tested from 32 to 64 to find the optimal *k*-value using abyss-pe script. The quality of microbial genomes was assessed using the bioinformatic tool CheckM 1.0.7 [19]. The genome sequences of closely related 10 species of the genus *Pseudomonas* were retrieved from the GenBank database. The average nucleotide identity (ANI) was calculated using the OrthoANI algorithm of the Chun lab's online ANI calculator [11]. In silico DNA–DNA hybridization (DDH) values were calculated by genome-to-genome distance calculator (GGDC) [15]. DNA G+C mol% was calculated from the genomic sequences.

Determination of Isoprenoid Quinones, Fatty Acids and Polar Lipids

For chemotaxonomic studies, cell biomass of strain ZYSR67-Z^T and the two closest reference strains were cultured in LB medium at 35 °C for late-logarithmic phase, and then lyophilized. Isoprenoid quinones were extracted as described by Komagata and Suzuki [8] and identified by HPLC as described by Minnikin et al. [17]. Fatty acid methyl esters were extracted as described by Kuykendall et al. [9] and analysed according to the instructions of the Microbial Identification System (MIDI, Microbial ID, USA) with the standard MIS Library Generation Software version 4.5. The mixed solution of chloroform/methanol/water (1:2:1, v:v:v) was used to extract polar lipids, which were separated on two-dimensional TLC (silica gel 60 F254 plate, 10 × 10 cm, Merck). Four kinds of sparing reagents were used to detect the corresponding lipids, comprising molybdophosphoric acid for total lipids, molybdenum blue for phospholipids, ninhydrin reagent for lipids containing free aminolipids and α-naphthol/sulphuric acid reagent for glycolipids [36].

Results and Discussion

Phenotypic Characterization

Cells of strain ZYSR67-Z^T were rod-shaped (0.3–0.5 µm in width and 1.0–1.2 µm in length) and motile with a single polar flagellum (Supplementary Fig. S1). Abundant growth was observed on both LB agar and Nutrient Agar (NA; BD Difco™) after incubation at 35 °C for 2 days. Strain ZYSR67-Z^T grew optimally at 35 °C, pH 6.0, and in the presence of 2.0% (w/v) NaCl. Other physiological and biochemical characteristics of strain ZYSR67-Z^T are included in the species description. All negative properties tested using API ZYM, API 20NE and API 50CH strips are presented in Supplementary Table S2.

A comparison of the physiological and biochemical characteristics of strains ZYSR67-Z^T, *P. guguanensis* JCM 18416^T and *P. alcaligenes* JCM 20561^T is shown in Table 1 and Supplementary Table S1. Several characteristics were found to discriminate strain ZYSR67-Z^T from *P. guguanensis* JCM 18416^T and *P. alcaligenes* JCM 20561^T. Especially, strain ZYSR67-Z^T was not able to produce H_2S, could not hydrolyse starch, casein, tyrosine and Tween 80, while two reference strains showed the contrary results. In addition, strain ZYSR67-Z^T could grow at 4 °C, which was much lower than the temperature range of reference strains *P. guguanensis* JCM 18416^T (20–42 °C) and *P. alcaligenes* JCM 20561^T (25–42 °C).

Genotypic Characterization

On the basis of 16S rRNA gene sequence alignment, strain ZYSR67-Z^T exhibited the highest similarity value (98.4%) with *P. guguanensis* JCM 18416^T and to other species of *Pseudomonas* including *P. alcaligenes* JCM 20561^T (98.1%), *P. indoloxydans* JCM 14246^T (98.2%), *P. alcaliphila* CGMCC 1.6415^T (97.9%) and *P. tuomuerensis* CGMCC 1.1365^T (97.2%), respectively. The phylogenetic trees reconstructed with all three treeing methods indicated that strain ZYSR67-Z^T formed an independent lineage within *Pseudomonas* and clustered with *P. guguanensis* JCM 18416^T and *P. alcaligenes* JCM 20561^T (Fig. 1), indicating that the strain represents a novel species within this genus. The overall topology of the phylogenetic tree based on individual or concatenated housekeeping genes (*gyrB, rpoD* and *rpoB*), also supported the conclusion obtained from 16S rRNA gene. MLSA analysis revealed that the *gyrB* gene sequence similarities between strain ZYSR67-Z^T and its closest phylogenetic neighbours were *P. guguanensis* JCM 18416^T (84.9%), *P. alcaligenes* JCM 20561^T (87.2%), *P. indoloxydans* JCM 14246^T (85.4%), *P. alcaliphila* CGMCC 1.6415^T (85.6%) and *P. tuomuerensis* CGMCC 1.1365^T (83.3%), respectively. The *rpoD* gene sequence similarities with the above 5 closest phylogenetic neighbour reference strains were 78.3, 80.2, 76.6, 76.7 and 79.7%, respectively. At the same time, the *rpoB* gene sequence similarity with the 5 reference strains were 92.7, 91.8, 91.8, 91.7 and 89.3%, respectively. The results obtained from the analysis of all these three genes are depicted as a consensus Maximum-likelihood phylogenetic tree (Supplementary Fig. S2), which was reconstructed based on the concatenated *gyrB* (596 bp), *rpoB* (471 bp) and *rpoD* (529 bp) gene sequences.

The draft genome of ZYSR67-Z^T was used to calculate the ANI and genome distance by computing in silico DDH with multiple type strains of species of the genus *Pseudomonas*. As shown in Table 2, the ANI values between strain ZYSR67-Z^T and the phylogenetically closely related 10 species of the genus *Pseudomonas* are 75.8–78.6%. The

Table 1 Differential characteristics of strain ZYSR67-Z^T and the type strains of its phylogenetic neighbours

Characteristics	1	2	3	4
Temperature (°C)	4–42	20–42[a]	25–42[a]	15–42
pH range	5.0–9.0	6.0–10.0[a]	6.0–10.0[a]	6.0–9.0
NaCl range (%, w/v)	0–5.0	0–7.0[a]	0–2.0[a]	0–6.5
Reduction of nitrate to				
Nitrite	+	−	+	+
N_2	+	−	−	ND
H_2S production	−	+	+	−
Hydrolysis of				
Starch	−	+	+	−
Gelatin	−	+	−	−
Casein	−	+	+	−
Tyrosine	−	+	+	ND
Tween 20	+	−	+	+
Tween 80	−	+	+	v
Urea	−	+	−	v
Enzymes(API ZYM)				
Alkaline phosphatase	+	+	+	−
Lipase (C14)	+	+	−	+
Cystine arylamidase	−	+	+	−
Trypsine	+	+	+	−
Chymotrypsin	−	+	−	−
API 20NE				
Assimilation of				
D-Glucose	−	+	−	−
D-Maltose	−	+	−	−
Potassium gluconate	−	+	−	−
Capric acid	−	+	+	v
Malic acid	+	+	−	v
Trisodium citrate	−	+	−	−
Phenylacetic acid	−	+	−	−
API 50CH				
Acid production from				
Glycerol	+	+	−	−
D-Ribose	+	−	−	−
D-Xylose	+	−	−	−
D-Galactose	+	−	−	−
D-Glucose	−	+	−	−
D-Fructose	+	+	−	−
D-Mannose	+	−	−	−
D-Maltose	+	+	−	−
D-Melibiose	+	−	−	−
D-Fucose	+	−	−	ND
DNA G+C content (mol%)	62.6	64.1	64.8	60.4

Strain: (1) ZYSR67-Z^T; (2) *P. guguanensis* JCM 18416^T; (3) *P. alcaligenes* JCM 20561^T; (4) *P. tuomuerensis* CGMCC 1.1365^T (Xin et al. [30]; Lin et al. [13]; Yang et al. [32]). Unless otherwise indicated, all data were obtained in this study; +, positive reaction; −, negative reaction; v, variable. All strains were positive for oxidase and catalase activities, hydrolysis of Tween 40, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities (API ZYM), assimilation of arginine dihydrolase (API 20NE). All trains were negative for indole production, fermentation of glucose, hydrolyse of esculin, assimilation of β-galactosidase, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and adipic acid (API 20NE)

[a]Date from Liu et al. [14]

in silico DDH values (the recommended results from formula 2) indicated that strain ZYSR67-Z^T and the closely related 10 type strains of the genus *Pseudomonas* shared only 21.4–23.1% DNA relatedness. The ANI values and in silico DDH values were strikingly lower than the threshold values of the species boundary (ANI 94–96% and in silico DDH 70%) [15, 21], revealing a low taxonomic relatedness between strain ZYSR67-Z^T and the other type strains of the *Pseudomonas* species. This result indicated that the strain ZYSR67-Z^T did not belong to any previously known species within the genus *Pseudomonas*. The genomic DNA G+C content was 62.6 mol%, within the range observed for species of the genus *Pseudomonas* [18].

Isoprenoid Quinones, Fatty Acids and Polar lipids Characterization

The predominant respiratory quinone detected in strain ZYSR67-Z^T was ubiquinone-9 (Q-9), which was consistent with most members in genus *Pseudomonas* [14]. Trace amount of Q-8 (7.9%) was also detected in strain $ZYSR67^T$, but not in *P. guguanensis* JCM 18416^T and *P. alcaligenes* JCM 20561^T. Fatty acid analysis of strain ZYSR67-Z^T revealed that $C_{18:1}$ $\omega 7c$ (11.9%), $C_{12:0}$ (12.5%), $C_{16:0}$ (25.0%) and summed features 3 ($C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH) (30.9%) were the major cellular fatty acids (> 10%). These data were similar with other two reference strains, and all the three strains possessed $C_{18:1}$ $\omega 7c$ and $C_{16:0}$ as the most abundant fatty acids (Table 3). However, the profiles of strain ZYSR67-Z^T were distinguishable from the reference strains by differences in the proportions of some fatty acids (Table 3). The polar lipids of strain ZYSR67-Z^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG), one unidentified aminophospholipid (APL1), one unidentified aminolipid (AL1), two unidentified phospholipids (PL1, PL2) and two unknown glycolipids (GL1, GL2) (Supplementary Fig. S3).

Based on the morphological, physiological, phylogenetic and chemotaxonomic characteristics as described above, strain ZYSR67-Z^T is considered to represent a novel species of the genus *Pseudomonas*, for which the name *P. pharmafabricae* sp. nov. is proposed.

Description of *Pseudomonas pharmafabricae* sp. nov.

Pseudomonas pharmafabricae (phar.ma.fab'ri.cae. L. masc. gen. n. *pharmafabricae* from a pharmaceutical factory).

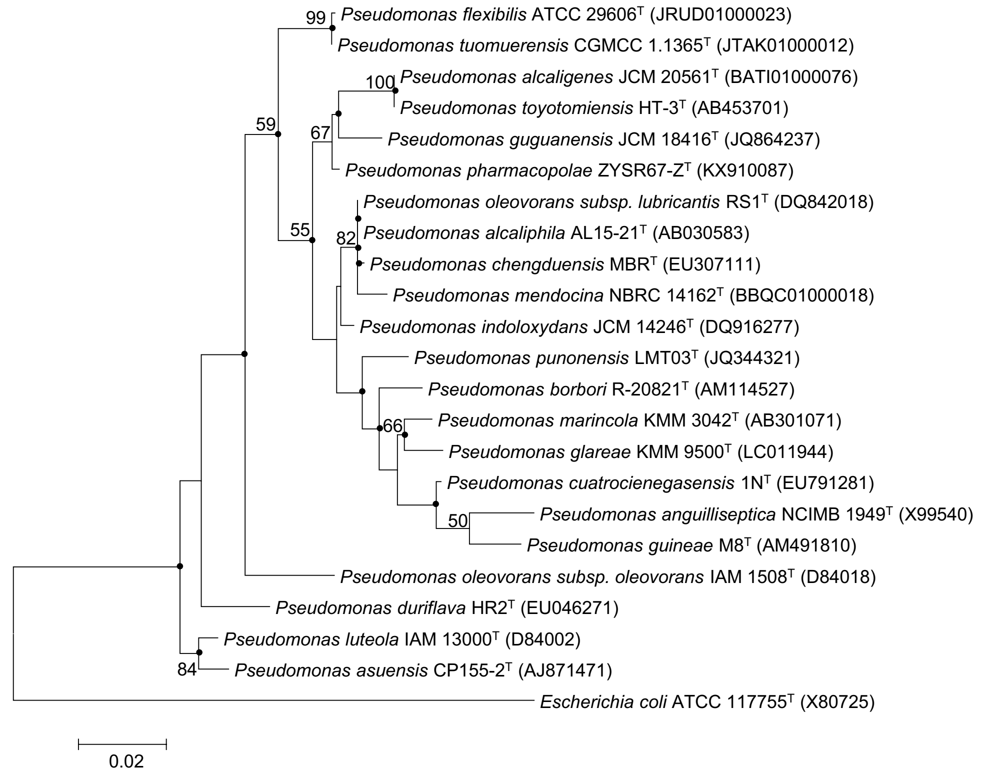

Fig. 1 Maximum-likelihood phylogenetic tree of strain ZYSR67-Z^{T} with the closely related taxa based on 16S rRNA gene sequences (1462 bp). Numbers at branching points represent bootstrap values (%) from 1000 replicates; only values ≥50% are shown. *Escherichia coli* ATCC 11775^{T} (X80725) was used as an outgroup. Solid circles indicate that the corresponding nodes were also recovered in neighbour-joining tree. Bar, 0.05 substitutions per nucleotide position

Table 2 Average nucleotide identities and in silico DNA–DNA hybridization (DDH) values calculated when comparing the genomic sequence of ZYSR67-Z^{T} with those of other species of the genus *Pseudomonas*

Compared species of genus *Pseudomonas*	OrthoANI values with ZYSR67-Z^{T}	In silico DDH values with ZYSR67-Z^{T}
P. guguanensis JCM 18416^{T}	77.91	22.60
P. alcaligenes JCM 20561^{T}	78.60	23.10
P. toyotomiensis HT-3^{T}	77.41	22.10
P. chengduensis MBR^{T}	77.48	22.30
P. oleovorans subsp. *lubricantis* RS1^{T}	77.72	22.10
P. alcaliphila CGMCC 1.6415^{T}	77.50	22.20
P. mendocina NBRC 14162^{T}	77.27	22.10
P. tuomuerensis CGMCC 1.1365^{T}	76.63	21.50
P. flexibilis ATCC 29606^{T}	76.65	21.50
P. punonensis LMT03^{T}	75.83	21.40

The following is the list of strains and their GenBank accession number for the genomic sequence in parentheses:

(1) *P. guguanensis* JCM 18416^{T} (FNJJ00000000); (2) *P. alcaligenes* JCM 20561^{T} (BATI00000000); (3) *P. toyotomiensis* HT-3^{T} (FOXK00000000); (4) *P. chengduensis* MBR^{T} (FMZQ00000000); (5) *P. oleovorans* subsp. *lubricantis* RS1^{T} (FNZC00000000); (6) *P. alcaliphila* CGMCC 1.6415^{T} (FNAE00000000); (7) *P. mendocina* NBRC 14162^{T} (BBQC00000000); (8) *P. tuomuerensis* CGMCC 1.1365^{T} (JTAK00000000); (9) *P. flexibilis* ATCC 29606^{T} (FMUP00000000); (10) *P. punonensis* LMT03^{T} (FRBQ00000000)

Cells are Gram-stain-negative, aerobic, rod-shaped (0.3–0.5 × 1.0–1.2 μm) and motile with single polar flagellum (Supplementary Fig. S1). Colonies are 2–3 mm in diameter, circular, canary yellow, semi-transparent with entire margins after incubation on LB agar at 35 °C for 2 days. Growth occurs at 4–42 °C (optimum, 35 °C), pH 5.0–9.0 (optimum, pH 6.0) and with 0–5% (w/v) NaCl (optimum, 2%). Catalase- and oxidase-positive. Positive results in tests for aerobic reduction of nitrate and nitrite, hydrolysis of Tween 20, Tween 40 and Tween 60. Sensitive to tobramycin, rifamycin, erythromycin, ofloxacin, kanamycin, doxycycline and ciprofloxacin. Negative results in tests for

Table 3 The cellular fatty acid contents (%) of strain ZYSR67-Z^T and the type strains of the reference relatives

Fatty acid	1	2	3
Straight-chain			
$C_{10:0}$	4.2	5.8	0.5
$C_{12:0}$	**12.5**	**10.6**	**13.6**
$C_{14:0}$	3.8	7.3	0.7
$C_{15:0}$	1.4	1.1	0.9
$C_{16:0}$	**25.0**	**25.1**	**24.2**
Cyclo			
$C_{17:0}$ cyclo	–	–	1.5
Unsaturated			
$C_{18:1}$ ω7c	**11.9**	**22.4**	**32.2**
Hydroxy			
$C_{10:0}$ 3-OH	3.6	7.5	3.9
$C_{12:0}$ 3-OH	3.8	6.7	4.5
Summed feature[a]			
3	**30.9**	9.2	**13.2**

Strain: 1, ZYSR67-Z^T; 2, *P. guguanensis* JCM 18416^T; 3, *P. alcaligenes* JCM 20561^T. –, not detected. Major fatty acids (> 10% of total fatty acids) are highlighted in bold. Fatty acids below 1% in all strains are deleted. All data are from this study

[a]Summed features groups of one or more fatty acids that could not be separated by GLC with the MIDI System. Summed feature 3 comprises $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH;

Voges–Proskauer, methyl red, hydrolysis of starch, gelatin, casein, tyrosine, Tween 80 and urea. Indole and H_2S are not produced. Resistant to ampicillin, nystatin, bacitracin, vancomycin and clindamycin. In API ZYM tests, production of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), naphthol-AS-BI-phosphohydrolase, valine arylamidase, trypsin, acid phosphatase and leucine arylamidase are positive reactions. In API 20NE tests, positive results are obtained for activity of arginine dihydrolase and assimilation of malic acid. In API 50CH tests, acid is produced from glycerol, D-ribose, D-xylose, D-galactose, D-fructose, D-mannose, esculin ferric citrate, D-maltose, D-melibiose, D-fucose, potassium gluconate and potassium 5-ketogluconate. In GN2 MicroPlates analysis, positive results were observed for utilization of pyruvic acid methyl ester, acetic acid, β-hydroxybutyric acid, D,L-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-glutamic acid, L-alaninamide, D-alanine, L-alanine, L-asparagine, L-histidine, hydroxy-L-proline, L-proline, inosine and putrescine. The predominant isoprenoid quinone is Q-9, and presence of trace amounts of Q-8. The major fatty acids (> 10%) are $C_{12:0}$, $C_{16:0}$, $C_{18:1}$ $\omega 7c$ and summed features 3 ($C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH). The polar lipids are PE, PG, DPG, one unidentified aminophospholipid (APL), one unidentified aminolipid (AL), two unidentified phospholipids (PL1, PL2) and two unknown glycolipids (GL1, GL2). The DNA G+C content of the type strain is 62.6 mol%.

The type strain, ZYSR67-Z^T (= CGMCC 1.15498^T = JCM 31306^T) was isolated from pharmaceutical wastewater sample collected in Jinhua, Zhejiang province, China.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Claus D (1992) A standardized gram staining procedure. World J Microb Biot 8:451–452
2. Ding LX, Yokota A (2002) Phylogenetic analysis of the genus *Aquaspirillum* based on 16S rRNA gene sequences. FEMS Microbiol Lett 212:165–169
3. Dong XZ, Cai MY (2001) Determinative manual for routine bacteriology, 1st edn. Scientific Press, Beijing (**English translation**)
4. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
5. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Biol 20:406–416
6. Hugh R, Ikari P (1964) The proposed neotype strain of *Pseudomonas alcaligenes* monlas 1928. Int Bull Bacteriol Nomencl Taxon 14:103–107
7. Jin Y, Gan G, Yu X et al (2017) Isolation of viable but nonculturable bacteria from printing and dyeing wastewater bioreactor based on resuscitation promoting factor. Curr Microbiol 74:787–797
8. Komagata K, Suzuki KI (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207
9. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE (1998) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobiurn japonicum*. Int J Syst Bacteriol 38:358–361
10. Lang E, Burghartz M, Spring S, Swiderski J, Sproer C (2010) *Pseudomonas benzenivorans* sp. nov. and *Pseudomonas saponiphila* sp. nov., represented by xenobiotics degrading type strains. Curr Microbiol 60:85–91
11. Lee I, Kim YO, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103
12. Li SH, Jin Y, Cheng J, Park DJ, Kim CJ, Hozzein WN, Wadaan MAM, Shu WS, Ding LX, Li WJ (2014) *Gordonia jinhuaensis* sp. nov., a novel actinobacterium, isolated from a VBNC (viable but non-culturable) state in pharmaceutical wastewater. Antonie Van Leeuwenhoek 106:347–356
13. Lin SY, Hameed A, Liu YC, Hsu YH, Lai WA, Chen WM, Shen FT, Young CC (2013) *Pseudomonas sagittaria* sp. nov., a siderophore-producing bacterium isolated from oil-contaminated soil. Int J Syst Evol Microbiol 63:2410–2417

14. Liu YC, Young LS, Lin SY, Hameed A, Hsu YH, Lai WA, Shen FT, Young CC (2013) *Pseudomonas guguanensis* sp. nov., a gammaproteobacterium isolated from a hot spring. Int J Syst Evol Microbiol 63:4591–4598
15. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60
16. Migula W (1894) Über ein neues System der Bakterien. Arb Bakteriol Inst Karlsruhe 1:235–238
17. Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An Integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
18. Palleroni NJ (2005) Pseudomonas. In: Brenner DJ, Krieg NR, Staley JT (eds) Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer, New York, pp 323–379
19. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055
20. Peix A, Ramrez-Bahena MH, Velázquez E (2009) Historical evolution and current status of the taxonomy of genus *Pseudomonas*. Infect Genet Evol 9:1132–1147
21. Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131
22. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
23. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I (2009) ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123
24. Su XM, Liu YD, Hashmi MZ, Hu JX, Ding LX, Wu M, Shen CF (2015) *Rhodococcus biphenylivorans* sp. nov., a polychlorinated biphenyl-degrading bacterium. Antonie Van Leeuwenhoek 107:55–63
25. Sun C, Pan J, Zhang XQ, Su Y, Wu M (2015) *Pseudoroseovarius zhejiangensis* gen. nov., sp nov., a novel alpha-Proteobacterium isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov. and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae*. comb. nov.. Antonie Van Leeuwenhoek 108:291–299
26. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526
27. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729
28. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
29. Wang MQ, Sun L (2016) *Pseudomonas oceani* sp. nov., isolated from deep seawater. Int J Syst Evol Microbiol 66:4250–4255
30. Xin YH, Zhang DC, Liu HC, Zhou HL, Zhou YG (2009) *Pseudomonas tuomuerensis* sp. nov., isolated from a bird's nest. Int J Syst Evol Microbiol 59:139–143
31. Yamamoto S, Harayama S (1998) Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of gyrB, rpoD and 16S rRNA genes. Int J Syst Bacteriol 48:813–819
32. Yang G, Han L, Wen J, Zhou S (2013) *Pseudomonas guangdongensis* sp. nov., isolated from an electroactive biofilm, and emended description of the genus *Pseudomonas* Migula 1894. Int J Syst Evol Microbiol 63:4599–4605
33. Yong YC, Wu XY, Sun JZ, Cao YX, Song H (2015) Engineering quorum sensing signaling of *Pseudomonas* for enhanced wastewater treatment and electricity harvest: a review. Chemosphere 140:18–25
34. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617
35. Yu XY, Zhang L, Ren B, Yang N, Liu M, Liu XT, Zhang LX, Ding LX (2015) *Arthrobacter liuii* sp. nov., resuscitated from Xinjiang desert soil. Int J Syst Evol Microbiol 65:896–901
36. Zhang XQ, Wu YH, Zhou X, Zhang X, Xu XW, Wu M (2016) *Parvularcula flava* sp. nov., an alphaproteobacterium isolated from surface seawater of the South China Sea. Int J Syst Evol Microbiol 66:3498–3502
37. Zhong ZP, Liu Y, Hou TT, Liu HC, Zhou YG, Wang F, Liu ZP (2015) *Pseudomonas salina* sp. nov., isolated from a salt lake. Int J Syst Evol Microbiol 65:2846–2851