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Pseudomonas donghuensis sp. nov., exhibiting high-yields of siderophore

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Abstract A strain giving high-yields of siderophores, designated HYS^T, was isolated from the water of East Lake (also called Donghu Lake) of Wuhan in China. Strain HYS^T is Gram-stain negative, non-spore-forming and rod-shaped with polar flagella. Phylogenetic analysis based on 16S rRNA gene and the other three housekeeping genes (*gyrB*, *rpoD* and *rpoB*) indicated that strain HYS^T belongs to the genus *Pseudomonas*. Genomic DNA comparison experiments including DNA–DNA hybridization and whole-genome sequence similarities were performed between HYS^T and its phylogenetically most closely related type strains, all of the relatedness values are lower than the threshold to ascribe strain HYS^T to a known species. The major cellular fatty acids of strain HYS^T are $C_{16:0}$, $C_{17:0\ cyclo}$, Summed feature 3 ($C_{16:1\ \omega7c}$ or/and $C_{16:1\ \omega6c}$) and Summed feature 8 ($C_{18:1\ \omega7c}$ or $C_{18:1\ \omega6c}$). Its predominant isoprenoid quinone was identified as Q-9, and the minor isoprenoid quinone was Q-8. Phylogenetic analysis together with genomic DNA comparison, phenotypic metabolic tests and chemotaxonomic analysis justified the proposal of strain HYS^T as a representative of a novel species, for which the name *Pseudomonas donghuensis* sp. nov. is proposed. The type strain is HYS^T (= CCTCC AB 2012141^T = NRRL B-59108^T).

Keywords *Pseudomonas donghuensis* · Taxonomy · Bacteria · Novel species

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Introduction

Siderophore are a group of small molecular compounds that chelate ferric iron (Neilands 1995). Microorganisms synthesize and secrete siderophores to acquire iron, because iron is highly insoluble in aerobic environments (Ferguson et al. 1998). Microorganisms which synthesize siderophores with high affinity of iron can be used as biocontrol agency to inhibit plant pathogenic fungi and bacteria by iron competition (Sayye and Chincholkar 2009). Strain HYS^T was isolated from the water of East Lake (also called Donghu Lake) of Wuhan in China, and was named by its 'high-yielding of siderophore' phenotype as determined by a universal siderophore detection

Table 1 Accession numbers and correspondent references of gene sequences used for phylogenetic analysis in this study

Species	16S rRNA gene	*GyrB* gene	*RpoD* gene	*RpoB* gene	References
Cellvibrio japonicus Ueda107	NC_010995:				DeBoyRT et al. 2008
	807042-808575	3980-6400	899813-901714	821813-825898	
P. abietaniphila^T	AJ011504	FN554166	FN554447	AJ717416	Ait Tayeb et al. 2005Mohn et al. 1999; Mulet et al. 2010
P. aeruginosa^T	X06684	AB039386	AB039607	AJ717442	Ait Tayeb et al. 2005; Toschka et al. 1988; Yamamoto et al. 2000
P. agarici^T	AKBQ01000002	AB039457	AB039563	AJ717477	Ait Tayeb et al. 2005; Yamamoto et al. 2000
P. alcaliphila^T	AB030583	FN554167	FN554448	AJ717463	Ait Tayeb et al. 2005; Mulet et al. 2010; Yumoto et al. 2001
P. anguilliseptica^T	AB021376	FN554168	FN554449	FN554726	Anzai et al. 2000; Mulet et al. 2010
P. asplenii^T	AB021397	AB039455	AB039593	AJ717432	Ait Tayeb et al. 2005; Anzai et al. 2000; Yamamoto et al. 2000
P. baetica^T	FM201274	HE800470	FN678357	HE800504	López et al. 2012; Mulet et al. 2012b
P. chengduensis^T	EU307111	JX042516	JX042517	JX042515	Tao et al. 2014
P. chlororaphis^T	Z76673	FJ652718	AB039549	FJ652691	Mavrodi et al. 2010; Yamamoto et al. 2000
P. cichorii^T	Z76658	AB039434	AB039526	AJ717418	Ait Tayeb et al. 2005; Moore et al. 1996; Yamamoto et al. 2000
P. cremoricolorata^T	AB060137	FN554181	FN554462	AJ717476	Ait Tayeb et al. 2005; Mulet et al. 2010; Uchino et al. 2001
P. donghuensis^T	AJJP01000212: 2926-4463	AJJP01000074: 11425-13842	AJJP01000029: 6295-8142	AJJP01000184: 7678-11751	Gao et al. 2012
P. entomophila^T	NC_008027:				Vodovar et al. 2006
	115507-117024	4346-6766	435570-437420	502974-507047	
P. ficuserectae^T	AB021378	AB039418	AB039501	AJ717457	Ait Tayeb et al. 2005; Anzai et al. 2000; Yamamoto et al. 2000
P. fulva^T	D84015	AB039395	AB039586	AJ717419	Ait Tayeb et al. 2005; Anzai et al. 1997; Yamamoto et al. 2000
P. fuscovaginae^T	FJ483519	FN554185	FN554467	AJ717433	Ait Tayeb et al. 2005; Mulet et al. 2010.
P. graminis^T	Y11150	FN554187	FN554469	AJ717429	Ait Tayeb et al. 2005; Behrendt et al. 1999; Mulet et al. 2010
P. guariconensis^T	HF674459	HF674462	HF674460	HF674461	Toro et al. 2013
P. japonica^T	AB126621	GQ996725	HE577795	HE577800	Mulet et al. 2012a; Pungrasmi et al. 2008
P. jessenii^T	AF068259	FN554191	FN554473	AJ717447	Ait Tayeb et al. 2005; Mulet et al. 2010; Verhille et al. 1999
P. lini^T	AY035996	FN554196	FN554478	AJ717466	Ait Tayeb et al. 2005; Delorme et al. 2002; Mulet et al. 2010
P. lutea^T	AY364537	FN554198	FN554480	FN554738	Mulet et al. 2010; Peix et al. 2004
P. mendocina^T	Z76664	AJ633103	AJ633567	AJ279967	Cladera et al. 2004; Lorenz and Sikorski 2000; Moore et al. 1996
P. mohnii^T	AM293567	AM293561	FN554487	FN554741	Camara et al. 2007; Mulet et al. 2010
P. monteilii^T	AF064458	FN554205	FN554488	AJ717455	Ait Tayeb et al. 2005; Elomari et al. 1997; Mulet et al. 2010
P. moorei^T	AM293566	AM293560	FN554489	FN554742	Camara et al. 2007; Mulet et al. 2010
P. moraviensis^T	AY970952	FN554206	FN554490	FN554743	Mulet et al. 2010; Tvrzová et al. 2006
P. mosselii^T	AF072688	FN554207	FN554491	FN554744	Mulet et al. 2010
P. oleovorans^T	AF094735	AB039396	AB039601	AJ717461	Ait Tayeb et al. 2005; Yamamoto et al. 2000
P. oryzihabitans^T	AM262973	FN554210	FN554494	AJ717470	Ait Tayeb et al. 2005; Mulet et al. 2010; Tvrzová et al. 2006
P. parafulva^T	AB060132	FN554216	FN554500	AJ717471	Ait Tayeb et al. 2005; Mulet et al. 2010; Uchino et al. 2001
P. plecoglossicida^T	AB009457	FN554218	FN554503	AJ717456	Ait Tayeb et al. 2005; Mulet et al. 2010; Nishimori et al. 2000

Table 1 continued

Species	16S rRNA gene	*GyrB* gene	*RpoD* gene	*RpoB* gene	References
P. putida^T	NC_021505: 164022-165561	4197-6617	495867-497717	495867-497717	Ohji et al. 2014
P. reinekei^T	AM293565	AM293559	FN554508	FN554754	Camara et al. 2007; Mulet et al. 2010
P. rhizosphaerae^T	AY152673	FN554224	FN554510	FN554755	Mulet et al. 2010; Peix et al. 2003
P. straminea^T	D84023	AB039410	AB039600	FN554758	Anzai et al. 1997; Mulet et al. 2010; Yamamoto et al. 2000
P. taiwanensis^T	EU103629	FJ418634	HE577796	HE577797	Mulet et al. 2012a; Wang et al. 2010
P. toyotomiensis^T	AB453701	AB494447	AB548145	AB548147	Hirota et al. 2011
P. vancouverensis^T	AJ011507	FN554232	FN554517	AJ717473	Ait Tayeb et al. 2005; Mohn et al. 1999; Mulet et al. 2010
P. vranovensis^T	AY970951	AUED01000002: 512272-514689	AUED01000002: 48939-50789	AUED01000032: 3807-7880	Tvrzová et al. 2006
P. xanthomarina^T	AB176954	AM905836	AM905872	FN554765	Mulet et al. 2008, 2010; Romanenko et al. 2005

assay (Gao et al. 2012; Yu et al. 2014). Strain HYS^T inhibited growth of the plant pathogenic bacteria *Xanthomonas campestris* pv. *Badrii* on LB medium which suggested strain HYS^T is a biocontrol agency candidate (unpublished).

For the convenience of analysis of siderophores and biocontrol related characteristics, the genomic DNA of strain HYS^T has been sequenced, and thereby generated a 5,639,475 bp draft genome sequence (Gao et al. 2012). By searching multiple nucleotide and protein databases, 4,556 of 4,955 total predicted protein coding genes found homologs with several strains of genus *Pseudomonas,* therein 1,856 genes, 1,191 genes and 1,059 genes shared highest similarity with strains of *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas entomophila* respectively. The results indicated distinct taxonomic standing of strain HYS^T despite its close relationship with species of the genus *Pseudomonas.* Here, we have performed the multilocus phylogenetic analysis based on four house keeping genes together with genomic DNA sequence comparative analysis, phenotypic and chemotaxonomic analyses in order to assess the taxonomic affiliation of strain HYS^T.

Materials and methods

Bacterial strains

Chrome azurol S (CAS) medium described by Neilands (1995) was used for the isolation of strain HYS^T from the samples of water of East Lake of Wuhan in China. Strain HYS^T was grown well on iron-poor modified King's B medium (Yu et al. 2014) and Luria–Bertani (LB) medium. Reference strains *P. putida* ATCC 12633^T, *Pseudomonas vranovensis* DSM 16006^T, *Pseudomonas fuscovaginae* DSM 7231^T and *Pseudomonas asplenii* DSM 17133^T were obtained from American Type Culture Collection (ATCC) and German Collection of Microorganisms and Cell Cultures (DSMZ) respectively. All strains were grown on LB medium at 28 °C.

Phylogenetic analysis

The 16S rRNA sequence of strain HYS^T was retrieved from its genome, and then compared with sequences of

16S rRNA gene sequences of the type strains database from EzTaxon server (Chun et al. 2007). Species which have similarity higher than 97.00 % with strain HYS^T were used for multilocus sequence analysis (MLSA). Three more species, *Pseudomonas aeruginosa* DSM 50071^T, *Pseudomonas anguilliseptica* NCMB 1949^T and *Pseudomonas straminea* IAM 1598^T were also included in the analysis. *Cellvibrio japonicus* Ueda107 was used as outgroup. The accession numbers of sequences used for MLSA and correspondent references are shown in Table 1.

A series of individual trees of the 16S rRNA, gyrase beta subunit (*gyrB*), beta subunit of the RNA polymerase (*rpoB*) partial genes, sigma 70 subunit of RNA polymerase partial genes (*rpoD*) as well as concatenated gene tree of these four genes were constructed following the methods described in Mulet et al. (2008, 2010). Phylogenetic analysis was performed using the software package MEGA version 6.0 (Tamura et al. 2013) after multiple alignment of the data via CLUSTALW (Larkin et al. 2007). Phylogenetic trees were constructed by neighbour-joining (NJ) (Saitou and Nei 1987) algorithms and evolutionary distances were calculated with Jukes–Cantor method (Jukes and Cantor 1969) and the topology of the NJ tree was evaluated by bootstrap analysis on the basis of 1,000 replications (Felsenstein 1985).

Genome comparison

DNA–DNA hybridization experiments were performed between strain HYS^T and the type strain of the phylogenetically most closely related *P. putida* ATCC 12633^T, *P. vranovensis* DSM 16006^T, *P. fuscovaginae* DSM 7231^T and *P. asplenii* DSM 17133^T, using the microplate method as described elsewhere (Ezaki et al. 1989; Xie and Yokota 2003).

Average nucleotide identity (ANI) values as the index of whole-genome sequence similarity were calculated as described by Goris et al. (2007). The genomic sequence from one of the genomes in a pair ('query') was cut into consecutive 1,020 nt fragments, and then used to search against the whole genomic sequence of the other genome in the pair ('the reference') by using the BLASTN algorithm (Altschul et al. 1997). The ANI between the query genome and the reference genome was calculated as the mean identity of all BLASTN matches that showed more than 30 % overall sequence identity over an alignable region of at least 70 % of their length. Therefore, only homologous DNA fragments were considered in the calculations. Reverse searching, i.e., in which the reference genome is used as the query, was also performed to provide reciprocal value.

Morphological, physiological and biochemical characterization

Cell morphology was examined using phase-contrast (Olympus BX51) and transmission electron (FEI Tecnai G2) microscopes (TEM) using cells grown for 12 h. Gram staining of the cells was carried out according to the Gram staining procedure described by Doetsch (1981). Growth at different temperatures (4, 16, 22, 30, 37, 45 and 50 °C) was investigated for 2 days, with growth assessed based on the occurrence of visible colonies on agar. Salt tolerance was tested on modified LB medium in which the NaCl concentration (w/v) was adjusted to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 % respectively. Fluorescent pigment formation was observed on King medium B (King et al. 1954). Hydrolysis of gelatin was investigated according to Stanier et al. (1966). Additional physiological and biochemical characteristics were determined using API 20NE and Biolog GN2 MicroPlate according to the manufacturers' instructions. All tests were performed using the reference strains in one experiment.

Chemotaxonomic characterization

For respiratory quinones identification, cells were lyophilized and respiratory quinones component were extracted following the method of Collins et al. (1977). Samples were analyzed by HPLC as described by Xie and Yokota (2003).

For analysis of cellular fatty acids, 2 mg late-logarithmic phase cells of strain HYS^T and the reference strains were collected. The methods used for harvesting, saponification, methylation and extraction of cellular fatty acids followed the protocols of the Sherlock Microbial Identification System (MIDI) version 6.0. Separation and identification of fatty acid methyl esters was performed using a Agilent 6890N

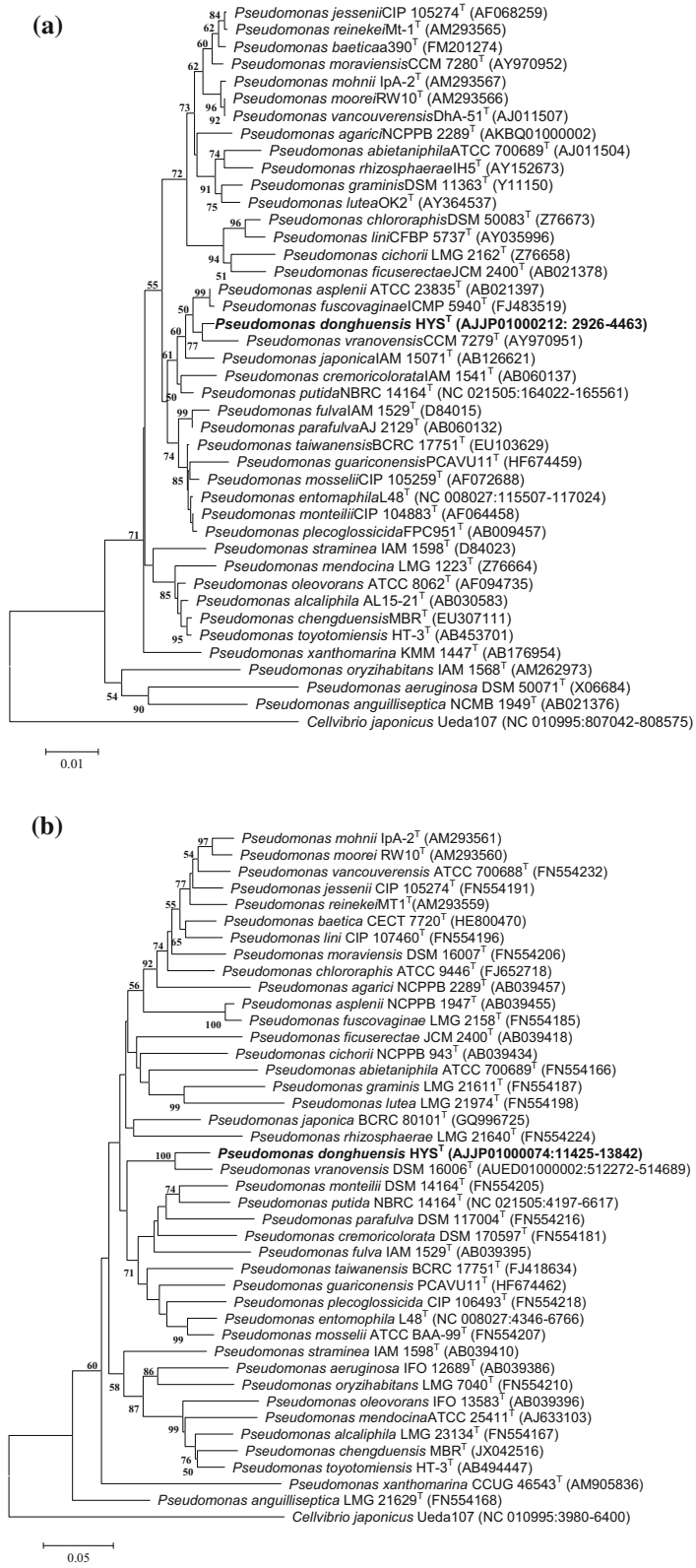
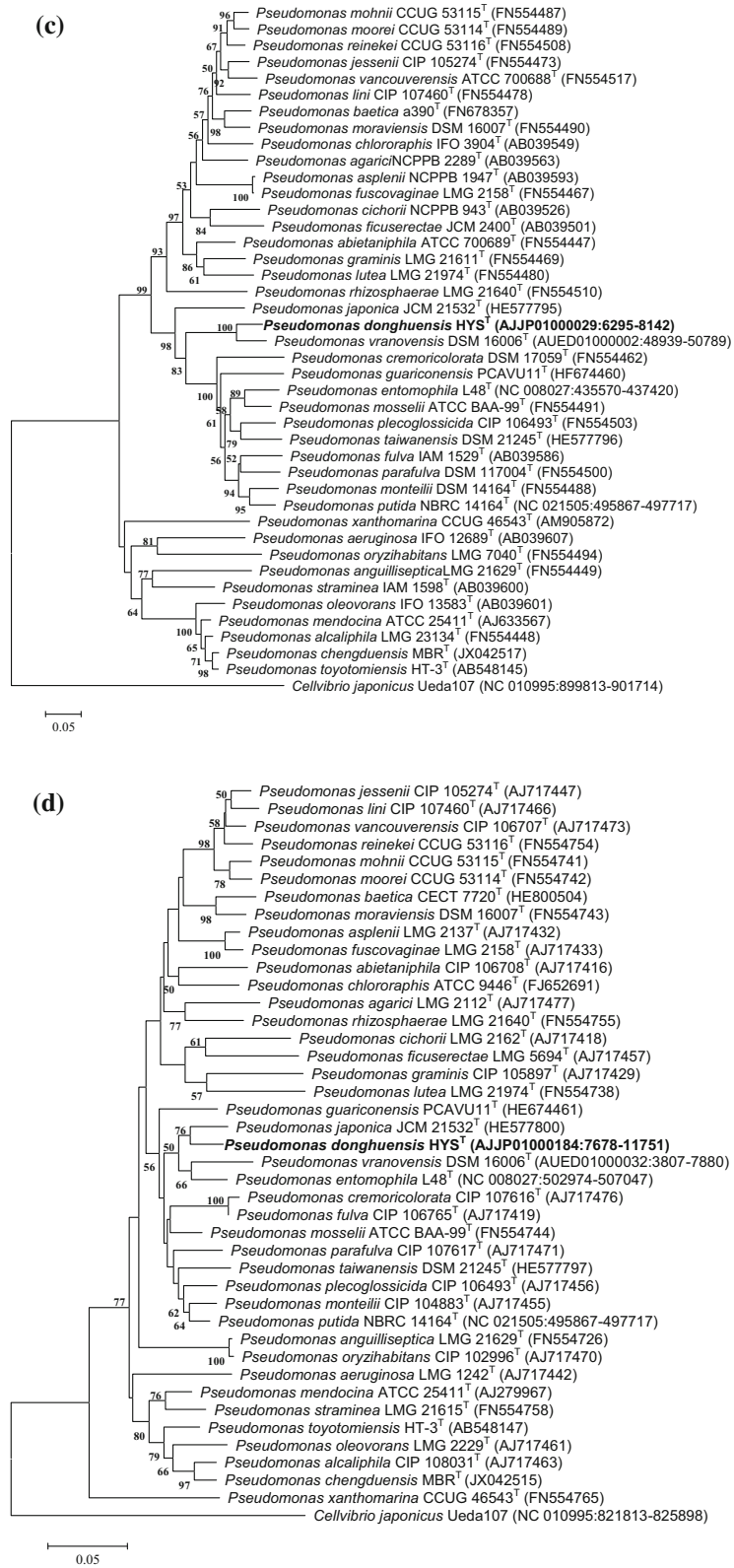


Fig. 1 Neighbour-joining phylogenetic trees based on **a** the 16S rRNA gene; **b** the *gyrB* gene; **c** the *rpoD* genes; **d** the *rpoB* gene; and **e** four genes concatenated. Distance matrices were calculated by the Jukes–Cantor method. Bootstrap values (1,000 replications) are shown as percentages at each node only if they are 50 % or greater

Fig. 1 continued



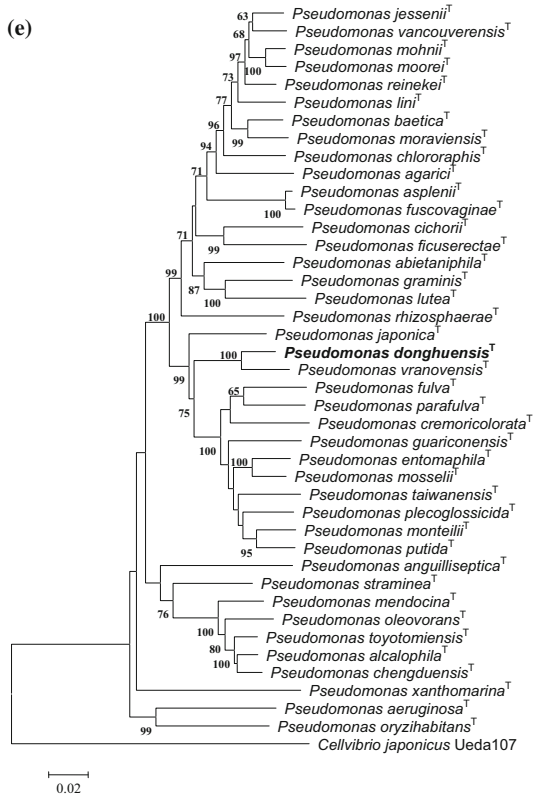


Fig. 1 continued

gas chromatograph, with MIDI Sherlock TSBA6 (version of the database) (Sasser 1990).

Results and discussion

Phylogenetic analysis

The 1,538 nt length 16S rRNA sequence of strain HYS^T was used as query sequence to search the type strain 16S rRNA gene sequences database using

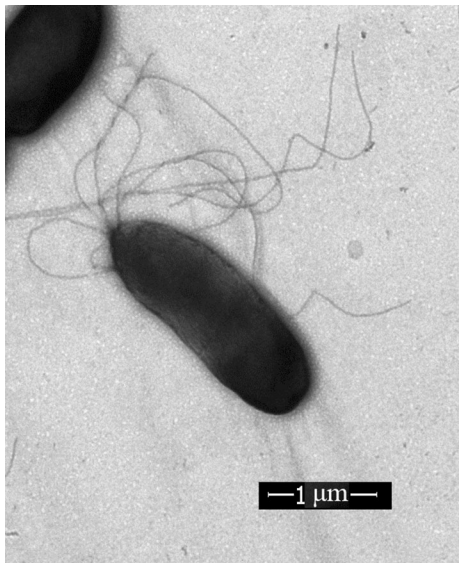


Fig. 2 Transmission electron microscopy of a negatively stained cell of *Pseudomonas donghuensis* HYS^T, showing polar flagella as observed in an FEI Tecnai G2 electron microscope

EzTaxon server. There were thirty-seven type strains of *Pseudomonas* species sharing similarity higher than 97 % with strain HYS^T, which represented species of different intrageneric groups except group *P. aeruginosa*, *P. anguilliseptica* and *P. straminea* (Mulet et al. 2010). In order to reconfirm the taxonomic standing of strain HYS^T within the genus *Pseudomonas*, the thirty-seven species together with *P. aeruginosa* DSM 50071^T, *P. anguilliseptica* NCMB 1949^T and *P. straminea* IAM 1598^T were employed in MLSA of housekeeping core genes as recommended for new pseudomonas species description (Mulet et al. 2010). Following the approach of Mulet et al., a series of individual phylogenetic trees of four genes (16S rDNA, *gyrB*, *rpoD* and *rpoB*) and four genes concatenated tree have been reconstructed as shown in

Table 2 DNA–DNA hybridization relatedness values and gene sequence similarities between *strain* HYS^T and closely related *Pseudomonas* species

Bacterial strains	Reassociation (%) with labelled DNA from		Strain HYS^T gene sequence similarities (%)	
	HYS^T	*P. vranovensis* DSM 16006^T	16S rRNA	MLSA
P. asplenii DSM 17133^T	50	51	98.91	90.57
P. donghuensis HYS^T	100	58	100.00	100.00
P. fuscovaginae DSM 7231^T	46	49	98.98	90.48
P. putida ATCC 12633^T	52	43	99.06	91.70
P. vranovensis DSM 16006^T	58	100	98.99	95.47

Table 3 Characteristics that differentiate strain HYS^T and closely related *Pseudomonas* species

Characteristics	1	2	3	4	5
Fluorescein	+	+	−	+	+
Hydrolysis of gelatin	+	−	−	−	+
Activity of enzymes (API 20NE)					
Reduction of nitrate	+	−	+	−	−
Cytochrome oxidase	+	−	−	+	+
Assimilation (API 20NE)					
Phenylacetic acid	+	+	−	−	−
Utilization of carbon sources (BIOLOG GN2)					
Tween 40	+	+	−	−	w
L-Fucose	−	−	+	−	w
D-Galactose	−	+	−	+	+
Sucrose	+	−	−	−	−
D-Galacturonic acid	−	+	−	+	+
D-Glucuronic acid	−	+	−	+	+
p-Hydroxy Phenylacetic acid	+	+	−	+	+
Malonic acid	−	+	−	+	+
D-Saccharic acid	−	+	−	+	+
Succinamic acid	+	w	−	−	−
Glucuronamide	−	+	−	+	+
Glycyl-L-Glutamic acid	+	+	−	+	+
L-Pyroglutamic acid	+	−	+	+	+
Inosine	+	+	−	+	+
Uridine	+	+	−	+	w
Phenylethylamine	−	+	−	−	−

1 HYS^T, 2 *P. putida* ATCC 12633^T, 3 *P. vranovensis* DSM 16006^T, 4 *P. fuscovaginae* DSM 7231^T, 5 *P. asplenii* DSM 17133^T

+ Positive; − negative; *w* weakly positive

Fig. 1. The highest gene sequence similarity of four genes concatenated analysis was found to be 95.47 % between strain HYS^T and *P. vranovensis* DSM 16006^T, and similarities between strain HYS^T and other reference type strains are lower than 91.70 %. Since the recommended threshold value for species discrimination is 97 % (Goris et al. 2007), strain HYS^T is suggested to represent a novel species of the genus *Pseudomonas*.

Genome comparisons

DNA–DNA hybridization experiments were performed between strain HYS^T and *P. putida* ATCC

Table 4 Cellular fatty acid composition of strain HYS^T and reference strains

Fatty acid	Percentage composition in strains				
	1	2	3	4	5
10:0	1.75	0.35	–	–	0.06
10:0 3-OH	8.33	5.14	6.04	4.91	4.39
11:0 3-OH	0.13	–	0.07	–	–
12:0	1.66	2.98	3.61	1.97	1.43
12:0 2-OH	5.3	6.4	4.85	7.00	6.95
12:0 3-OH	5.94	4.92	4.97	5.76	5.43
12:1 3-OH	2.21	0.43	1.04	–	–
14:0	0.59	0.59	0.89	0.82	0.53
15:1 iso G	–	0.11	–	–	–
15:0 iso	–	0.2	–	–	0.06
15:0 anteiso	–	–	–	–	0.27
16:0	27.53	26.64	27.1	32.54	27.21
16:0 3-OH	–	0.17	0.24	–	–
16:1 ω5c	–	–	0.1	–	0.11
17:0	–	0.16	0.14	–	0.21
17:1 ω8c	–	0.15	0.14	–	0.14
17:1 anteiso ω9c	–	–	0.08	–	–
17:0 cyclo	11.29	9	5.64	1.02	3.14
17:1 iso ω5c	0.07	–	–	–	–
18:0	0.47	0.33	0.19	0.67	0.25
18:1 ω9c	0.49	–	–	–	–
19:0 iso	0.11	–	–	–	–
19:0 cyclo ω8c	0.62	0.36	–	–	–
19:0 10-methyl	–	0.27	0.3	–	–
Summed feature					
1	0.42	–	–	–	–
2	0.36	–	0.1	–	–
3	21.34	27.57	31.64	36.22	40.11
5	–	0.23	–	–	–
8	11.4	13.99	12.86	9.09	9.72

Summed features are combinations of fatty acids that cannot be separated by the MIDI system. Summed feature 1 contains $C_{13:0\ 3\text{-}OH}$ and/or iso-$C_{15:1\ H}$; summed feature 2 contains $C_{12:0}$ aldehyde; summed feature 3 contains $C_{16:1\ \omega6c}$ and/or $C_{16:1\ \omega7c}$; summed feature 5 contains anteiso-$C_{18:0}$ and/or $C_{18:2\ \omega6,\ 9c}$; summed feature 8 contains $C_{18:1\ \omega7c}$ or $C_{18:1\ \omega6c}$

All data obtained in this study as described in Materials and method section

1 HYS^T, 2 *P. putida* ATCC 12633^T, 3 *P. vranovensis* DSM 16006^T, 4 *P. fuscovaginae* DSM 7231^T, 5 *P. asplenii* DSM 17133^T

– Not detected

12633^T, *P. vranovensis* DSM 16006^T, *P. fuscovaginae* DSM 7231^T and *P. asplenii* DSM 17133^T. The results are shown in Table 2. The highest DNA–DNA hybridization value was found to be 58 % between strain HYS^T and *P. vranovensis* DSM 16006^T, which is lower than the threshold 70 % threshold value routinely applied for species discrimination (Wayne et al. 1987).

Calculation of ANI of whole-genome sequences is an approach offered by Goris et al. (2007) to accurately replace DNA–DNA hybridization for strains for which genome sequences are available. Recently it is reported that the recommended cut-off value of 70 % DDH for species delineation is corresponded to 95 % ANI (Kim et al. 2014). According to the MLSA and DNA–DNA hybridization data, strain HYS^T and *P. vranovensis* DSM 16006^T are most closely related. Therefore ANI of whole-genome sequences has been calculated. Since *P. putida* ATCC 12633^T shared the highest similarity with strain HYS^T based 16S rRNA sequence, the ANI between these two strains is also calculated. The ANI of strain HYS^T to *P. vranovensis* DSM 16006^T and *P. putida* ATCC 12633^T are 85.25 and 80.96 %, respectively. Both of the ANI values are lower than the 95 %, thus confirming separate taxonomic standing of strain HYS^T.

Morphological, physiological, and biochemical characteristics

Strain HYS^T was determined to be Gram-stain negative, non-spore-forming rods (approximately 0.6–1.1 μm wide and 1.7–2.5 μm long) with polar flagella as shown in Fig. 2. Colonies on LB agar are circular and smooth, and the diameter is about 2 mm after 24 h cultivation. Growth was found to occur at 4–37 °C and 0–5 % (w/v) of NaCl. Differential biochemical characteristics of strain HYS^T are listed in Table 3. Among those characteristics, hydrolysis of gelatin and utilization of sucrose could be typical feature to distinguish HYS^T from its closest phylogenetic relative species.

Chemotaxonomic characteristics

The predominant isoprenoid quinone was identified as Q-9, and the minor isoprenoid quinone as Q-8. This isoprenoid quinone composition is typical for the genus *Pseudomonas* (Collins and Jones 1981). The major cellular fatty acids were identified as $C_{16:0}$, $C_{17:0}$ cyclo, Summed feature 3 ($C_{16:1\ \omega7c}$ or/and $C_{16:1\ \omega6c}$) and Summed feature 8 ($C_{18:1\ \omega7c}$ or $C_{18:1\ \omega6c}$) as defined by the MIDI system. The fatty acid compositions of strain HYS^T and most closely related phylogenetic neighbours are shown in Table 4. Some qualitative and quantitative differences in fatty acid contents are observed between strain HYS^T and studied species. The proportions of $C_{10:0}$ and $C_{12:1\ 3\text{-OH}}$ was found to be significantly higher in strain HYS^T compared to those in references strains.

Conclusion

On the basis of the results of phylogenetic analysis based on 16S rRNA gene sequence similarities, MLSA, genomic comparison, phenotypic and chemotaxonomic data presented in this study, it can be concluded that strain HYS^T represents a new species of the genus *Pseudomonas*, for which the name *Pseudomonas donghuensis* sp. nov. is proposed.

Description of *Pseudomonas donghuensis* sp. nov.

Pseudomonas donghuensis (N. L. fem. adj. dong. hu' ensis, pertaining to Donghu, where the type strain was isolated).

Cells are Gram-negative, non-spore-forming rods (approximately 0.6–1.1 μm wide and 1.7–2.5 μm long) with polar flagella. Colonies on LB agar are circular and smooth, and the diameter is about 2 mm after 24 h cultivation. Growth occurs at 4–37 °C. NaCl concentrations for growth are 0–5 % (w/v). Fluorescein is produced on King B medium oxidase positive. Gelatin is hydrolyzed. According to API 20NE reduction of nitrate is reduced, D-glucose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Positive for Biolog GN2 MicroPlate substrates: Tween 40, Tween 80, *N*-Acetyl-D-Glucosamine, L-Arabinose, D-Fructose, α-D-Glucose, Sucrose, Methyl pyruvate, mono-Methyl-Succinate, Acetic acid, Cis-Aconitic acid, Citric acid, Formic acid, D-Gluconic acid, α-Hydroxy, Butyric acid, β-Hydroxy, Butyric acid, p-Hydroxy phenylacetic acid, α-Keto glutaric acid, α-Keto valeric acid, D,L-Lactic acid, Propionic acid,

Quinic acid, Succinic acid, Bromo succinic acid, Succinamic acid, L-Alaninamide, D-Alanine, L-Alanine, L-Alanyl-glycine, L-Asparagine, L-Aspartic acid, L-Glutamic acid, Glycyl-L-Glutamic acid, L-Histidine, Hydroxy-L-Proline, L-Leucine, L-Ornithine, L-proline, L-Pyroglutamic acid, D-Serine, L-Serine, L-Threonine, D,L-Carnitine, γ-Amino Butyric acid, Urocanic acid, Inosine, Uridine, Putrescine, 2-Aminoethanol and Glycerol. Major isoprenoid quinone is Q-9, and the minor isoprenoid quinone is Q-8. The major cellular fatty acids are $C_{16:0}$, $C_{17:0\ cyclo}$, Summed feature 3 ($C_{16:1\ \omega7c}$ or/and $C_{16:1\ \omega6c}$) and Summed feature 8 ($C_{18:1\ \omega7c}$ or $C_{18:1\ \omega6c}$).

The type strain is HYS^T (= CCTCC AB 2012141^T - = NRRL B-59108^T).

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