Pseudoalteromonas donghaensis sp. nov., isolated from seawater

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A Gram-negative, rod-shaped, motile and aerobic bacterium, designated strain HJ51^T, was isolated from a seawater sample from the East Sea, near South Korea. The isolate grew slowly at 4 °C, was able to grow at 40 °C, required NaCl and grew optimally at pH 6.5–7.0. The G+C content of the genomic DNA was 41.8 mol%. The major fatty acids were summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and summed feature 7 (C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain HJ51^T belonged to the genus *Pseudoalteromonas* and had 91.7–98.9% 16S rRNA gene sequence similarity with type strains of species of the genus *Pseudoalteromonas*. Strain HJ51^T had 7.2% DNA–DNA relatedness with *Pseudoalteromonas mariniglutinosa* DSM 15203^T and 12.9% with *Pseudoalteromonas prydzensis* DSM 14232^T. On the basis of the phenotypic, phylogenetic and genomic data, strain HJ51^T represents a novel species of the genus *Pseudoalteromonas*, for which the name *Pseudoalteromonas donghaensis* sp. nov. is proposed. The type strain is HJ51^T (=KCTC 22219^T=LMG 24469^T).

The genus *Pseudoalteromonas* was separated from the genus *Alteromonas*, which was originally described by Baumann *et al.* (1972), on the basis of comparative 16S rRNA gene sequence analysis (Gauthier *et al.*, 1995). Many members of the genus *Pseudoalteromonas* have been isolated from marine environments and the genus is one of largest within the class *Gammaproteobacteria*. The abundance and adaptation of members of the genus *Pseudoalteromonas*, through the production of hydrolytic enzymes such as lipase, chitinase, agarase, amylase and protease, mean that they have an important role in their habitats (Ivanova *et al.*, 2003). Members of the genus *Pseudoalteromonas* also produce many bioactive compounds and have important interactive roles with marine fauna and flora (Bowman, 2007; Thomas *et al.*, 2008). At the time of writing, the genus *Pseudoalteromonas* comprises 34 recognized species and two subspecies (Euzéby, 1997). In the course of our screening programme for biopolymer-degrading bacteria from deep seawater, a novel strain, designated HJ51^T, was isolated from the East Sea, near South Korea.

Seawater samples from the East Sea, 6 km from the coast (38° 21' 25" N 128° 35' 30" E) and at depths of 200–500 m,

were collected by the Deep Ocean Water Application Research Center from July 2005 to June 2006. Samples were stored at 4 °C in darkness for several months until required for bacterial isolation. For isolation of strain HJ51^T, 200 µl seawater was serially diluted and spread on 1/10 marine agar 2216 (MA; Difco) and incubated at 25 °C for 7 days. Single colonies were purified by repeated streaking on MA. Strain HJ51^T was grown routinely in marine broth 2216 (MB; Difco) and YPS broth or on MA and YPS agar. YPS medium contained (l⁻¹) 1 g yeast extract, 5 g peptone and 20 g NaCl (15 g agar). Cultures were stored at –80 °C in MB with 25% (v/v) glycerol. The reference strains *Pseudoalteromonas mariniglutinosa* DSM 15203^T (Romanenko *et al.* 2003) and *Pseudoalteromonas prydzensis* DSM 14232^T (Bowman, 1998) were obtained from the DSMZ (Braunschweig, Germany).

Morphology and cell size were determined by phase-contrast microscopy (80i; Nikon). Gram-staining was performed with BD Gram-stain kits according to the instructions of the manufacturer and the non-staining method as described by Buck (1982). Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activities were determined using oxidase reagent (bioMérieux), according to the manufacturer's instructions, after cultivation for 2 days. Hydrolysis of casein, starch, Tween 80 and agar was tested as described by Smibert & Krieg (1994), using MA as the basal medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HJ51^T is FJ754319.

A supplementary table is available with the online version of this paper.

Hydrolysis of chitin was determined on MA supplemented with 1.0% soluble chitin and detection of DNase was performed on DNase test agar (Difco) supplemented with 2.0% NaCl. Acid production from glucose, single-carbon-source assimilation and additional physiological characteristics were determined using the API 20NE, API ID32 GN and API ZYM galleries (bioMérieux), according to the manufacturer's instructions, except that cells were suspended in 2% (w/v) NaCl solution. Growth at 0, 4, 10, 15, 20, 25, 30, 35, 37, 40 and 45 °C, at pH 5.0–10.0 (at intervals of 0.5 pH unit) and with 0–14% (w/v) NaCl was determined on YPS agar for 14 days at 25 °C and with 2% NaCl unless otherwise stated.

Strain HJ51^T was shown to be Gram-negative with both tests. Cells were aerobic, motile rods (0.4–0.6 × 1.5–3.5 µm), which were within the limits previously described for members of the genus *Pseudoalteromonas*. Colonies on MA after 3 days were smooth, slimy, non-pigmented, translucent and 4.0–6.0 mm in diameter. Strain HJ51^T was oxidase- and catalase-positive and grew with 1–13% (w/v) NaCl (optimum 2% NaCl); no growth occurred without NaCl. Strain HJ51^T grew at 4–40 °C (optimum 25–30 °C); no growth occurred at 0 °C or 45 °C. Strain HJ51^T grew at pH 5.5–9.5 (optimum 6.5–7.0). Strain HJ51^T could not only hydrolyse casein, DNA and starch, as can most of its close phylogenetic neighbours, but also it could weakly hydrolyse agar and chitin, which are rarely degraded by members of the genus *Pseudoalteromonas*, and aesculin and gelatin. Reduction of nitrates to nitrites and acidification of glucose by strain HJ51^T were observed. Strain HJ51^T was discriminated from its closest phylogenetic neighbours, *P. mariniglutinosa* DSM 15203^T and *P. prydzensis* DSM 14232^T, by having positive reactions for assimilation of L-histidine, 3-hydroxybutyrate, acetate, D-mannose and trisodium citrate and production of α-chymotrypsin, α-fucosidase and urease (Table 1). Phenotypic comparison of strain HJ51^T with other members of the genus *Pseudoalteromonas* is provided in Supplementary Table S1 (available in IJSEM Online).

For cellular fatty acid analysis, strain HJ51^T and the reference strains were cultivated for 3 days on MA. The fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) and analysed by GC (6890; Hewlett Packard) using the Microbial Identification software (Sasser, 1990). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in *n*-hexane/water (1:1, v/v). The crude quinone in *n*-hexane was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analysed by HPLC, as described by Hiraishi *et al.* (1996). For the measurement of the G+C content of the chromosomal DNA, genomic DNA was extracted and enzymically degraded into nucleosides and the G+C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC.

Table 1. Phenotypic characteristics of strain HJ51^T and closely related type strains of species of the genus *Pseudoalteromonas*

Taxa: 1, HJ51^T; 2, *P. mariniglutinosa* DSM 15203^T; 3, *P. prydzensis* DSM 14232^T. All data were taken from this study except where indicated. All strains were positive for assimilation of L-arabinose, D-glucose, maltose, sucrose, propionate, N-acetylglucosamine, L-proline and L-serine, production of catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-mannosidase, agarase (weakly), amylase, caseinase, chitinase (weakly) and DNase and hydrolysis of aesculin and gelatin. All strains were negative for Gram-staining, reduction of nitrates to nitrogen, assimilation of L-fucose, melibiose, D-ribose, inositol, D-sorbitol, caprate, citrate, itaconate, malonate, suberate, valerate, 3-hydroxybenzoate, 4-hydroxybenzoate, 5-ketogluconate, potassium gluconate, adipic acid and phenylacetic acid and production of indole, lipase (C14), cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase and arginine dihydrolyase. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Growth at 40 °C	+	–	–
Reduction of nitrates to nitrites	+	–	–
Acid production from glucose	+	–	+
Production of:			
α-Chymotrypsin	+	–	–
α-Fucosidase	+	–	–
α-Glucosidase	w	–	w
Urease	+	–	–
Assimilation of:			
Acetate	+	–	–
L-Alanine	+	–	+
Glycogen	w	–	+
L-Histidine	+	–	–
3-Hydroxybutyrate	+	–	–
2-Ketogluconate	–	–	+
DL-Lactate	–	+	–
Malic acid	–	w	+
Mannitol	–	+	–
D-Mannose	+	–	–
Rhamnose	–	+	–
Salicin	–	+	–
Trisodium citrate	+	–	–
DNA G+C content (mol%)*	41.8	40.3	38–39

*Data for *P. mariniglutinosa* DSM 15203^T and *P. prydzensis* DSM 14232^T were taken from Romanenko *et al.*, (2003) and Bowman (1998), respectively.

The cellular fatty acid contents of strain HJ51^T and the two reference strains are shown in Table 2. Strain HJ51^T contained summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 30.66%), C_{16:0} (23.17%) and summed feature 7 (C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t; 23.01%) as the major fatty acids and significant amounts of C_{12:0} 3-OH (7.34%). This fatty acid composition was in accordance

Table 2. Fatty acid contents of strain HJ51^T and closely related type strains of species of the genus *Pseudoalteromonas*

Strains: 1, HJ51^T; 2, *P. mariniglutinosa* DSM 15203^T; 3, *P. prydzensis* DSM 14232^T. All data were taken from this study. Fatty acids comprising <0.5% in all strains are not shown. —, Not detected/not reported; ECL, equivalent chain-length.

Fatty acid (%)	1	2	3
Saturated			
C _{12:0}	1.76	2.23	2.26
C _{14:0}	1.21	1.10	1.60
C _{15:0}	—	0.27	0.35
C _{16:0}	23.17	29.66	32.31
C _{17:0}	0.83	0.92	0.55
C _{18:0}	2.90	3.19	2.38
Unsaturated			
C _{16:1} ω9c	0.53	0.48	0.40
C _{17:1} ω7c	1.23	—	—
C _{17:1} ω8c	—	1.04	0.66
C _{18:1} ω9c	1.42	1.26	1.07
C _{20:1} ω9t	0.65	0.29	—
C _{20:4} ω6,9,12,15c	0.84	—	—
Branched-chain			
iso-C _{12:0} 3-OH	0.71	0.31	0.53
iso-C _{16:0}	0.48	—	0.37
Hydroxy			
C _{10:0} 3-OH	0.56	0.48	0.43
C _{12:0} 3-OH	7.34	7.87	8.41
C _{12:1} 3-OH	—	0.26	—
C _{16:0} 3-OH	0.52	0.47	0.57
Summed features*			
3	0.76	0.45	0.49
4	30.66	32.32	33.30
7	23.01	15.28	12.15
C _{19:0} 10-methyl	—	0.41	0.37
Unknown ECL 11.80	1.42	0.71	1.79

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{14:0} 3-OH and/or iso-C_{16:1} I. Summed feature 4 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 consisted of one or more of C_{18:1}ω7c, C_{18:1}ω9t and C_{18:1}ω12t. The double bond position indicated by a capital letter is unknown.

with closely related members of the genus *Pseudoalteromonas*. However, the proportions of C_{16:0} and summed feature 7 were different between strain HJ51^T and the reference strains. In addition, small amounts of C_{17:1}ω7c and C_{20:4}ω6,9,12,15c were found only in strain HJ51^T. Strain HJ51^T contained ubiquinone-8 as the major respiratory lipoquinone. The DNA G+C content was 41.8 mol%.

For phylogenetic analysis, genomic DNA was extracted using a genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified using the universal bacterial primer set 27F and 1492R and the purified amplification products were sequenced by Solgent (Park *et al.*, 2006). A

full sequence (1506 bp) was obtained using SeqMan (DNASTAR) and compiled with 16S rRNA gene sequences of related taxa obtained from GenBank. Multiple alignment was performed using CLUSTAL_X (Thompson *et al.*, 1997) and gaps were edited using BioEdit (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were constructed using neighbour joining (Saitou & Nei, 1987) with MEGA3 (Kumar *et al.*, 2004) and maximum likelihood (Felsenstein, 1981) with PHYLIP (Felsenstein, 1993). Bootstrap values were calculated using 1000 replications (Felsenstein, 1985).

Phylogenetic analysis of the 16S rRNA gene sequence showed that strain HJ51^T was a member of the genus *Pseudoalteromonas*. The neighbour-joining and maximum-likelihood trees revealed that strain HJ51^T clustered with *Pseudoalteromonas lipolytica* LMEB 39^T (Xu *et al.*, 2010), *P. mariniglutinosa* KMM 3635^T (Romanenko *et al.*, 2003) and *P. prydzensis* ACAM 620^T (Bowman, 1998) (Fig. 1). Strain HJ51^T was most similar to *P. lipolytica* LMEB 39^T (98.9% 16S rRNA gene sequence similarity) and *P. mariniglutinosa* KMM 3635^T (98.1%), and showed lower levels of similarity to *P. prydzensis* ACAM 620^T (97.4%) and the other type strains included in the analysis (91.7–96.8%).

DNA–DNA hybridization between strain HJ51^T and the reference strains was performed using photobiotin-labelled DNA probes and micro-dilution wells as described by Ezaki *et al.* (1989). Hybridization was performed with five replications for each sample, the highest and lowest values were excluded and the mean of the remaining three values was quoted as the DNA–DNA relatedness value.

Currently in bacterial systematics, species are defined if they show <70% DNA–DNA relatedness (Wayne *et al.*, 1987) and organisms that have <97% 16S rRNA gene sequence similarity will not show >60% DNA–DNA relatedness, regardless of the hybridization method employed (Stackebrandt & Goebel, 1994). However, with the genera *Pseudoalteromonas* and *Amycolatopsis*, the cut-off value for DNA–DNA relatedness should be 99.0% (Romanenko *et al.*, 2003; Wink *et al.*, 2003). DNA–DNA relatedness values between strain HJ51^T and *P. mariniglutinosa* DSM 15203^T and *P. prydzensis* DSM 14232^T, which showed >97% 16S rRNA gene similarity, were 7.2% and 12.9%, respectively, which clearly indicated that strain HJ51^T represented a novel species.

Therefore, on the basis of phenotypic, chemotaxonomic and genotypic data, strain HJ51^T should be classified as representing a novel species of the genus *Pseudoalteromonas*, for which the name *Pseudoalteromonas donghaensis* sp. nov. is proposed.

Description of *Pseudoalteromonas donghaensis* sp. nov.

Pseudoalteromonas donghaensis (dong.ha.en'sis. N.L. fem. adj. *donghaensis* of or belonging to Donghae, the Korean name for the East Sea, from which the type strain was isolated).

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain HJ51^T within the genus *Pseudoalteromonas*. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered with the maximum-likelihood method. *Alginicola bacteriolytica* IAM 14595^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Cells are Gram-negative, motile, aerobic rods, 0.4–0.6 µm wide and 1.5–3.5 µm long. Colonies are smooth, mucoidal, translucent and non-pigmented, 4.0–6.0 mm in diameter, on MA. Oxidase- and catalase-positive. NaCl is essential for growth; growth is observed with 1–13% (w/v) NaCl (optimum 2–3% NaCl). Growth occurs slowly at 4 °C, but not at 45 °C (optimum 25–30 °C). Growth occurs at pH 5.5–9.5 (optimum pH 6.5–7.0). Reduces nitrate to nitrites, but not to nitrogen. Produces acid from glucose. Does not produce indole. Hydrolyses aesculin, casein, DNA, gelatin and starch and weakly hydrolyses chitin and agar. Produces alkaline phosphatase, urease, protease, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-fucosidase, α-glucosidase (weakly), N-acetyl-β-glucosaminidase and α-mannosidase, but not lipase (C14), cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase or arginine

dihydrolase. Utilizes L-arabinose, D-glucose, maltose, D-mannose, sucrose, L-alanine, L-histidine, L-proline, L-serine, acetate, trisodium citrate, propionate, 3-hydroxybutyrate, N-acetylglucosamine and glycogen as sole carbon sources, but not L-fucose, melibiose, rhamnose, D-ribose, salicin, inositol, mannitol, D-sorbitol, caprate, citrate, itaconate, DL-lactate, malonate, suberate, valerate, 2-ketogluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, 5-ketogluconate, potassium gluconate, adipic acid, malic acid or phenylacetic acid. The predominant ubiquinone is Q-8. The major fatty acids are summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and summed feature 7 (C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t).

The type strain, HJ51^T (=KCTC 22219^T=LMG 24469^T), was isolated from seawater (depth 200–500 m) of the East Sea, near South Korea. The DNA G + C content of the type strain is 41.8 mol%.

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