Pedobacter oryzae sp. nov., isolated from rice paddy soil

Yeji Jeon,¹ Jeong Myeong Kim,² Jin Ho Park,¹ Se Hee Lee,² Chi-Nam Seong,³ Sang-Suk Lee⁴ and Che Ok Jeon²

Correspondence
Che Ok Jeon
cojeon@cau.ac.kr

¹Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

²Department of Life Science, Chung-Ang University, Seoul 156-756, Republic of Korea

³Department of Biology, Sunchon National University, Sunchon 540-742, Republic of Korea

⁴Department of Animal Science & Technology, Sunchon National University, Sunchon 540-742, Republic of Korea

A Gram-stain-negative, strictly aerobic bacterium, designated strain N7^T, was isolated from a rice paddy in South Korea. Cells of strain N7^T were non-motile, non-spore-forming rods. Growth was observed at 15–35 °C (optimum of 25–30 °C) and between pH 6.0 and 8.0 (optimum of pH 6.5–7.5). The predominant isoprenoid quinone was menaquinone-7. The major cellular fatty acids of strain N7^T were summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0} and iso-C_{16:0}. The G + C content of the genomic DNA was 37.7 mol%. Comparative 16S rRNA gene sequence analyses showed that strain N7^T formed a distinct phyletic line within the genus *Pedobacter*. Phylogenetic distances from strains of other *Pedobacter* species with validly published names were greater than 5.0 % (i.e. <95.0 % 16S rRNA gene sequence similarities). On the basis of phenotypic and molecular data, it is clear that strain N7^T represents a novel species within the genus *Pedobacter*, for which the name *Pedobacter oryzae* sp. nov. is proposed. The type strain is N7^T (=KACC 12821^T =DSM 19973^T).

Since the genus *Pedobacter*, which belongs to the family *Sphingobacteriaceae* of the phylum *Bacteroidetes*, was first proposed by Steyn *et al.* (1998) to reclassify two *Sphingobacterium* species and to accommodate two novel species, a large number of *Pedobacter* species have been isolated from various habitats such as soil (Ten *et al.*, 2006; Yoon *et al.*, 2007a, b, c, d), water (Gallego *et al.*, 2006; Muurholm *et al.*, 2007), a glacier (Shivaji *et al.*, 2005), sludge (Vanparys *et al.*, 2005) and the rhizosphere (Kwon *et al.*, 2007). In this study, taxonomic characterization is reported of strain N7^T, which was isolated from a rice paddy and is related phylogenetically to members of the genus *Pedobacter*.

Strain N7^T was isolated from rice paddy soil associated with the roots of *Oryza sativa* in Jinju (35° 19' N 128° 09' E), South Korea. Rice roots were sampled in September 2006, soil debris was removed from the roots by washing with sterile saline (0.9 %, w/v) and the suspension was

serially diluted using 0.9 % (w/v) saline. The different dilutions were spread on R2A agar (Difco) and incubated at 25 °C for 5 days. Subcultivation was done routinely on R2A agar aerobically at 30 °C for 3 days, except where indicated otherwise, and the bacterial isolate was maintained as a glycerol stock at –80 °C.

Amplification and sequencing of the 16S rRNA gene of strain N7^T was carried out as described by Kim *et al.* (2008). Briefly, a single colony of strain N7^T grown on R2A agar was resuspended in 100 µl of 5 % (w/v) Chelex-100 solution (Bio-Rad) and boiled for 10 min to prepare crude genomic DNA lysates. PCR amplification of 16S rRNA genes from the crude lysates was performed using the universal primers F1 (5'-AGAGTTTGATCMTGGCTCAG-3') and R13 (5'-TACGGYTACCTTGTTACGACTT-3') as described previously (Lu *et al.*, 2006). The resulting 16S rRNA gene sequence (1486 nt) was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to determine an approximate phylogenetic affiliation and aligned with sequences of closely related members of the genus *Pedobacter* by using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using three

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N7^T is EU109726.

A transmission electron micrograph of a cell of strain N7^T is available as supplementary material with the online version of this paper.

different algorithms, neighbour-joining, maximum-likelihood and maximum-parsimony, available in PHYLIP version 3.6 (Felsenstein, 2002). Resulting tree topologies were evaluated by bootstrap analysis based on 1000 resamplings. Sequence similarity values between the novel strain and other related bacteria were computed using the 'homology search' program in the EzTaxon server (<http://147.47.212.35:8080/>; Chun *et al.*, 2007). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain N7^T was most closely related to members of the genus *Pedobacter* within the family *Sphingobacteriaceae*, forming a distinct monophyletic lineage within this genus with a bootstrap value of 63 % (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were essentially the same as that of the neighbour-joining tree (not shown). Comparative 16S rRNA gene sequence analyses showed that strain N7^T was most closely related to *Pedobacter steynii* WB 2.3-45^T, *Pedobacter ginsengisoli* Gsoil 104^T, *Pedobacter caeni* DSM 16990^T and *Pedobacter africanus* DSM 12126^T, with respective 16S rRNA gene sequence similarities of 95.0, 94.9, 94.6 and 94.2 %. These values are well below the threshold generally accepted for species delineation (Stackebrandt *et al.*, 2002).

Growth was tested on laboratory-prepared Luria–Bertani (LB) agar and on R2A (Difco), tryptic soy (TSA; Difco) and nutrient (NA; Difco) agars at 30 °C.

Growth of strain N7^T at different temperatures and pH was examined by growing the isolate on R2A agar at 5–35 °C (at 5 °C intervals) and in R2A broth adjusted to pH 5.0–

10.0 (at 0.5 pH unit intervals). Media with different pH values were prepared using appropriate biological buffers; Na₂HPO₄/NaH₂PO₄ and Na₂CO₃/NaHCO₃ buffers were used below pH 8.0 and for pH 8.0–10.0, respectively (Gomori, 1955). Gram staining was performed using the bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology and the presence of flagella were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) with 2-day-old cells grown on R2A agar as described previously (Bernardet *et al.*, 2002; Jeon *et al.*, 2004). Gliding motility was observed by direct microscopic examination of the edge of colonies grown on R2A agar with 1 % (w/v) agar. Salt tolerance was tested on R2A agar supplemented with 0–3 % (w/v) NaCl (at 0.5 % intervals) for 5 days at 30 °C. Oxidase and catalase activities and production of indole and H₂S were determined according to the methods of Smibert & Krieg (1994). The production of flexirubin-type pigments and extracellular glycans was investigated using the KOH and Congo red tests, respectively, following the minimal standards for the description of new taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). Nitrate reduction was performed according to the method of Lányi (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Biochemical features were characterized by using the API 20NE kit as recommended by the manufacturer (bioMérieux) except that the kit was incubated for 3 days at 30 °C. Anaerobic growth was assessed under anaerobic (with 4–10 % CO₂) conditions using the GasPak Plus system (BBL) at 30 °C for 15 days. The following tests were performed in parallel on strain

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain N7^T and related taxa. Bootstrap values (percentages of 1000 replicates) greater than 50 % are shown. The sequence of *Cytophaga hutchinsonii* ATCC 33406^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

N7^T and on its closest relatives, *P. steynii* WB 2.3-45^T, *P. ginsengisoli* Gsoil 104^T, *P. caeni* DSM 16990^T and *P. africanus* DSM 12126^T. Antibiotic susceptibility tests were performed in duplicate using filter-paper discs (8 mm diameter; Whatman) containing the following antibiotics: ampicillin (10 µg), polymyxin B (100 U), streptomycin (50 µg), penicillin G (10 IU), gentamicin (30 µg), chloramphenicol (100 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg), carbenicillin (100 µg) and novobiocin (50 µg). Hydrolysis of CM-cellulose, casein, gelatin, Tweens 80 and 20, aesculin, urea, tyrosine and starch was investigated on R2A agar after 7 days incubation at 30 °C according to previously described methods (Lányi, 1987; Smibert & Krieg, 1994). Additional enzyme activities were characterized by using the API ZYM kit according to the instructions of the manufacturer (bioMérieux) except that the kit was incubated for 3 days at 30 °C.

Bacterial cells were Gram-stain-negative, straight rods (see Supplementary Fig. S1 in IJSEM Online). The phenotypic

features of strain N7^T are presented in Table 1 and in the species description. Some features are in accordance with the description of the genus *Pedobacter*, whereas others enable strain N7^T to be differentiated from closely related *Pedobacter* species (Table 1).

The whole-cell fatty acid compositions of strain N7^T and the four reference type strains (*P. steynii* WB 2.3-45^T, *P. ginsengisoli* Gsoil 104^T, *P. caeni* DSM 16990^T and *P. africanus* DSM 12126^T) were analysed according to the instructions of the Microbial Identification system (MIDI; Microbial ID) using cells grown on TSA at 30 °C for 3 days. Analysis of isoprenoid quinones was carried out using the methods described by Komagata & Suzuki (1987). The DNA G+C content of strain N7^T was determined using an HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). Strain N7^T contained menaquinone-7 as the major respiratory lipokinone (at a peak area ratio of approximately 98 %). The cellular fatty acids (>9 % of the total fatty acids) were

Table 1. Differential characteristics of strain N7^T and the type strains of related *Pedobacter* species

Strains: 1, strain N7^T (data from this study); 2, *P. steynii* WB 2.3-45^T (unless indicated, data from Muurholm *et al.*, 2007); 3, *P. caeni* DSM 16990^T (Vanparys *et al.*, 2005); 4, *P. ginsengisoli* Gsoil 104^T (Ten *et al.*, 2006; Yoon *et al.*, 2007b); 5, *P. africanus* DSM 12126^T (Steyn *et al.*, 1998; Yoon *et al.*, 2007b). All strains are positive for aerobic growth and for catalase, oxidase, alkaline phosphatase† and leucine arylamidase† activities. All strains are negative for Gram staining, sporulation, reduction of nitrate to nitrite, production of indole and H₂S, hydrolysis of CM-cellulose† and urease†, lipase (C14)† and α-fucosidase† activities. All strains are resistant to ampicillin† and gentamicin†. +, Positive; –, negative; w, weakly positive; NA, data not available.

Characteristic	1	2	3	4	5
Source of isolation	Soil	Water	Nitrifying inoculum	Soil	Soil, sludge
Colony colour*	P	IW/PW	CW/Y	WH	Y
Maximum growth temperature (°C)	35	30	37	30	37
Hydrolysis of:†					
Tyrosine	–	–	–	+	–
Starch	–	+	+	–	+
Casein	–	+	–	–	–
Gelatin	–	+	–	+	w
Tween 20	–	+	–	–	+
Tween 80	–	+	+	–	+
Aesculin	+	+	+	–	+
Enzyme activities (API ZYM):†					
Esterase (C4)	+	+	–	+	–
Valine arylamidase	+	+	–	+	–
Trypsin	w	+	–	–	–
α-Chymotrypsin	w	w	–	–	–
β-Glucosidase	+	+	–	–	–
N-Acetyl-β-glucosaminidase	–	+	–	+	+
Susceptibility to:†					
Tetracycline	+	+	+	+	–
Chloramphenicol	–	+	–	+	w
DNA G+C content (mol%)	37.7	NA	42.7	43.6	43.7–44.2

*CW, Creamish white; IW, ivory white; P, pink; PW, pearl white; WH, white; Y, yellow.

†Results from this study.

summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 10.8 %), iso-C_{15:0} (10.7 %), anteiso-C_{15:0} (10.1 %), C_{15:0} (9.5 %) and iso-C_{16:0} (9.5 %). Although the overall fatty acid profile of strain N7^T was similar to those of phylogenetically related species (Table 2), significant differences in the respective proportions of several components clearly distinguished strain N7^T (Vanparys *et al.*, 2005; Ten *et al.*, 2006). The G+C content of the genomic DNA of strain N7^T was 37.7 mol%. The fatty acid composition and major lipoquinone of strain N7^T were in accordance with those of members of the genus *Pedobacter* (Steyn *et al.*, 1998; Gallego *et al.*, 2006; Ten *et al.*, 2006), whereas the DNA G+C content of strain N7^T enabled it to be differentiated from related *Pedobacter* species (Table 1). Therefore, the data obtained in this polyphasic study support the description of strain N7^T as representative of a

novel species of the genus *Pedobacter*, for which the name *Pedobacter oryzae* sp. nov. is proposed.

Description of *Pedobacter oryzae* sp. nov.

Pedobacter oryzae (o.ry'zae. L. fem. n. *oryzae* of rice, referring to the rice paddy fields where the type strain was isolated).

Cells are Gram-stain-negative, non-motile, non-spore-forming, straight rods, 0.5–0.7 µm in diameter and 2.0–3.0 µm long when grown at 30 °C on R2A agar. Colonies on R2A agar are slightly raised, circular with regular edges and pink in colour. Growth occurs at 30 °C on R2A agar, LB, TSA and NA. Grows optimally on R2A agar without the addition of NaCl; growth is severely inhibited on R2A agar containing more than 2 % (w/v) NaCl. Growth on R2A agar occurs at 15–35 °C (optimum of 25–30 °C) and at pH 6.0–8.0 (optimum of pH 6.5–7.5). Anaerobic growth is not observed after 15 days at 30 °C on R2A agar. Oxidase- and catalase-positive. Nitrate is not reduced to nitrite. Congo red is not absorbed by colonies and flexirubin-type pigments are not produced. Aesculin is hydrolysed. Casein, gelatin, tyrosine, Tweens 80 and 20, urea, starch and CM-cellulose are not hydrolysed. Indole and H₂S are not produced. Acid is produced from D-mannose, but not from D-fructose, D-galactose, raffinose, D-glucose, myo-inositol, lactose, melibiose, D-mannitol, L-arabinose, arbutin or salicin. In the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, valine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase activities are present, but lipase (C14), β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Weak enzymic activities are observed for trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Trisodium citrate is assimilated, but D-glucose, D-mannose, N-acetylglucosamine, maltose, L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid are not assimilated (API 20NE). Resistant to polymyxin B, gentamicin, kanamycin, oleandomycin, neomycin, ampicillin, streptomycin, penicillin G, lincomycin, chloramphenicol, carbenicillin and novobiocin, but sensitive to tetracycline. The major isoprenoid quinone is menaquinone-7. The major cellular fatty acids (>9 % of the total fatty acids) are summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0} and iso-C_{16:0}.

The type strain is N7^T (=KACC 12821^T =DSM 19973^T), isolated from rice paddy soil in South Korea. The DNA G+C content of the type strain is 37.7 mol% (HPLC).

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Table 2. Cellular fatty acid compositions (%) of strain N7^T and type strains of related *Pedobacter* species grown on TSA

Strains: 1, strain N7^T; 2, *P. steynii* WB 2.3-45^T; 3, *P. caeni* DSM 16990^T; 4, *P. ginsengisoli* Gsoil 104^T; 5, *P. africanus* DSM 12126^T. All data are from this study. Fatty acids amounting to less than 1.0 % in all strains are not shown. tr, Trace amount (<1 %); –, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5
C _{14:0}	tr	1.1	3.9	1.1	1.6
C _{15:0}	9.5	tr	tr	tr	–
C _{16:0}	2.8	8.6	5.5	8.6	2.8
C _{17:0} cyclo	–	1.6	7.3	–	–
C _{15:0} 2-OH	1.1	tr	tr	1.5	–
C _{16:0} 3-OH	tr	2.4	2.4	1.4	–
C _{17:0} 2-OH	4.1	tr	tr	3.5	–
iso-C _{14:0}	1.4	–	tr	–	–
iso-C _{15:0}	10.7	20.4	18.6	20.7	40.5
iso-C _{16:0}	9.5	–	tr	tr	–
iso-C _{16:1} H	5.1	–	–	–	–
iso-C _{15:0} 3-OH	1.1	4.2	4.3	4.0	3.1
iso-C _{16:0} 3-OH	7.2	tr	tr	tr	–
iso-C _{17:0} 3-OH	5.9	10.5	9.1	10.9	12.6
anteiso-C _{11:0}	tr	1.2	tr	–	–
anteiso-C _{15:0}	10.1	tr	tr	1.8	1.3
C _{15:1} ω6c	6.6	–	tr	tr	–
C _{16:1} ω5c	tr	1.9	2.2	2.0	–
C _{17:1} ω6c	1.5	–	–	–	–
C _{17:1} ω8c	1.4	–	–	–	–
iso-C _{17:1} ω9c	2.7	1.8	1.8	3.0	7.8
anteiso-C _{17:1} ω9c	1.6	–	–	1.1	–
Summed feature 3*	10.8	39.4	38.1	30.8	26.8
Summed feature 4*	–	tr	tr	2.2	1.7
ECL 13.565	–	1.1	tr	1.5	2.0
ECL 13.961	–	–	1.4	–	–

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B.

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