Arenimonas donghaensis gen. nov., sp. nov., isolated from seashore sand

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A Gram-negative, aerobic bacterium, designated strain HO3-R19^T, which was isolated from seashore sand in Pohang city, Korea, was characterized on the basis of a polyphasic taxonomic approach. Phylogenetic analyses of 16S rRNA gene sequences revealed that strain HO3-R19^T represents a new lineage within the *Gammaproteobacteria*; sequence similarities between strain HO3-R19^T and members of other related genera were less than 93.5 %. Strain HO3-R19^T was also distinguished from related genera based on differences in several phenotypic characteristics. Cells were straight or slightly curved rods and formed white colonies on R2A agar. The major isoprenoid quinone was ubiquinone 8 (Q-8), and predominant cellular fatty acids were iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1ω9c}. The DNA G + C content of strain HO3-R19^T was 65.0 mol%. Based on physiological, biochemical and chemotaxonomic traits together with results of comparative 16S rRNA sequence analysis, strain HO3-R19^T is considered to represent a novel species in a new genus, for which the name *Arenimonas donghaensis* gen. nov., sp. nov. is proposed. The type strain of *Arenimonas donghaensis* is HO3-R19^T (=KACC 11381^T = DSM 18148^T).

The phylum *Proteobacteria* represents the largest phylogenetically coherent group of bacteria, comprising over 1300 recognized species (Garrity & Holt, 2001). During a study of bacterial diversity of oil-contaminated seashore sand by using a culture-dependent approach, a number of bacterial strains were isolated from sand samples. One of these strains, designated HO3-R19^T, forming yellowish-white colonies on R2A agar (Difco) after 3 days cultivation at 28 °C, was isolated from seashore sand in Pohang, Korea. In this study, we report the taxonomic characterization of strain HO3-R19^T, which was considered to represent a member of the class *Gammaproteobacteria*.

Strain HO3-R19^T was isolated via a dilution plating method onto R2A agar (Difco) after 7 days incubation at 28 °C. The resulting colonies were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days at 28 °C. Purified colonies were tentatively identified from partial 16S rRNA gene sequences.

Colony properties were also observed on R2A medium. Cell morphology was observed via light microscopy (AXIO; Zeiss) and transmission electron microscopy (model 912AB;

LEO) (Fig. 1). Flagellum type was also determined by transmission electron microscopy. Gram staining of strain HO3-R19^T was determined by using a Gram stain kit (Difco) according to the manufacturer's instructions. Phenotypic tests were performed by using standard procedures (Smibert & Krieg, 1994). Hydrolysis of carboxymethylcellulose (0.1 %, w/v), casein (5.0 %, w/v), starch (1.0 %, w/v), gelatin (0.4 %, w/v) and tyrosine (0.5 %, w/v) was tested as described by Lanyi (1987). Growth at different salinities was tested at 0, 2, 3, 5, 7 and 10 % NaCl (w/v) on R2A broth after cultivation for 7 days. Temperature range for growth was assessed at 4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C on R2A agar after 15 days incubation. For pH tests, R2A broth adjusted to initial pH values of 4, 5, 6, 7, 8, 9 and 10 with citrate/phosphate buffer or Tris/hydrochloride buffer (Breznak & Costilow, 1994) was used to examine the ability of the strain to grow at different pH for 15 days. For the nitrate and nitrite reduction tests, the isolate was inoculated into three serum bottles (25 ml) containing 13 ml R2A broth, while nitrate and nitrite were added as KNO₃ and NaNO₂ at concentrations of 10 mM. The reduction of nitrate and nitrite was monitored via an ion chromatograph (model IC-320; Dionex) equipped with a conductivity detector and anion exchange column (Metrosep Anion Supp 4; Metrohm).

Enzyme activities, acid production from carbohydrates and assimilation of carbon substrates were determined by using

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HO3-R19^T is DQ411038.

Fig. 1. Transmission electron micrograph of a cell of strain HO3-R19^T. Bar, 500 nm.

API 20NE and API ZYM test strips (bioMérieux) according to the manufacturer's instructions. API 20NE strips were read visually after 24 and 48 h incubation at 28 °C. For strain characterization, only values taken after 48 h were taken into account. API ZYM strips were read after 5 h incubation. Anaerobic growth was observed after incubation in an anaerobic chamber for 15 days at 28 °C on R2A agar. Strain HO3-R19^T was tested for its susceptibility to nine antimicrobial compounds by using the method described by Alves *et al.* (2003). The isolate was incubated on R2A plates with discs (bioMérieux) containing ampicillin (10 µg), erythromycin (30 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (15 µg), neomycin (30 µg), penicillin G (10 IU) or streptomycin (10 µg).

The following chemotaxonomic characteristics were analysed: isoprenoid quinone (as described by Groth *et al.*, 1996), fatty acids (according to the standard protocol of the

MIDI/Hewlett Packard Microbial Identification System; Sasser, 1990) and G + C content of the DNA (Mesbah *et al.*, 1989).

The 16S rRNA gene sequence of strain HO3-R19^T was analysed as described by Kwon *et al.* (2003). Phylogenetic analysis was performed by using the program MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data by using CLUSTAL W (Thompson *et al.*, 1994). Distances were obtained by using options according to the Kimura two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values from 1000 replications were used to obtain the confidence level of the branches (Felsenstein, 1985).

Comparative analysis of the 16S rRNA gene sequence with those of the type species of related genera indicated that strain HO3-R19^T was a member of the family *Xanthomonadaceae* and had a unique taxonomic position within the class *Gammaproteobacteria*. The tree constructed based on the neighbour-joining method indicated that strain HO3-R19^T formed a phyletic line that was distinct from those occupied by the type species of the closely related genera *Pseudoxanthomonas*, *Thermomonas*, *Silanimonas*, *Lysobacter*, *Luteimonas*, *Stenotrophomonas*, *Xylella* and *Xanthomonas* (Fig. 2). The topology of the phylogenetic tree based on the maximum-parsimony algorithm was similar to that of the tree constructed by using neighbour-joining analysis (data not shown). According to a BLAST search within the NCBI database, strain HO3-R19^T showed highest 16S rRNA gene sequence similarity to *Pseudoxanthomonas suwonensis* 4M1^T (93.5 %). Strain HO3-R19^T was also closely related to *Pseudoxanthomonas broegbernsis* DSM 12573^T and *Silanimonas lenta* 25-4^T, but with only 93.0 and 92.6 % 16S rRNA gene sequence similarity, respectively.

Cells of strain HO3-R19^T were aerobic, Gram-negative, motile, straight or slightly curved rods, 0.4–0.6 µm in width and 1.3–3.0 µm in length. One polar flagellum was observed

Fig. 2. Phylogenetic relationships between strain HO3-R19^T and related taxa on the basis of 16S rRNA gene sequences. The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987), and the 16S rRNA gene sequence of *Escherichia coli* ATCC 11775^T was used as the outgroup. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; values < 50 % are not indicated. Bar, 0.02 nucleotide substitutions per site.

under transmission electron microscopy, as with cells of other closely related taxa except *Lysobacter enzymogenes*. Colonies on R2A agar after 3 days incubation at 28 °C were yellowish white. Strain HO3-R19^T was positive for oxidase and catalase activities. It did not grow anaerobically. Strain HO3-R19^T grew in the temperature range 4–37 °C, with an optimum at 28 °C, contrasting markedly with *Thermomonas haemolytica*, *Stenotrophomonas maltophilia* and *Silanimonas lenta*. The pH for growth was in the range 7.0–9.0 with an optimum at pH 8.0. The strain also hydrolysed tyrosine, gelatin, DNA and casein. It tolerated up to 3 % NaCl (w/v). Strain HO3-R19^T was sensitive to erythromycin, fusidic acid, gentamicin, kanamycin, neomycin and streptomycin, and resistant to ampicillin, lincomycin and penicillin G.

The major respiratory lipoquinone of strain HO3-R19^T was ubiquinone 8 (Q-8), as with closely related taxa. After incubation for 3 days at 28 °C on R2A, the cellular fatty acids of strain HO3-R19^T included iso-C_{16:0} (31.0 %), iso-C_{15:0} (26.9 %), iso-C_{17:1}ω9c (14.7 %), iso-C_{14:0} (6.4 %), iso-C_{11:0} 3-OH (5.5 %), iso-C_{15:1} F (2.9 %), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω9c; 2.7 %), iso-C_{11:0} (2.2 %), summed feature 1 (C_{13:0} 3-OH and/or iso-C_{15:1} H; 1.8 %), iso-C_{16:1} H (1.2 %) and iso-C_{17:0} (1.1 %). The iso-branched fatty acids iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1}ω9c as major components are also characteristic of *T. haemolytica*, *Silanimonas lenta* and *Aquimonas voraii* but not of some other related genera. The predominant hydroxy fatty acid of strain HO3-R19^T was iso-C_{11:0} 3-OH, as is also the case for *Silanimonas lenta*, *T. haemolytica*, *Luteimonas mephitis*, *Lysobacter enzymogenes* and *A. voraii*. By contrast, some related taxa (*P. broegbernensis*, *Stenotrophomonas maltophilia*, *Xylella fastidiosa* and *Xanthomonas campestris*) contained hydroxy fatty acids that were not detected in strain HO3-R19^T (Table 1).

Strain HO3-R19^T contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as major polar lipids in addition to small amounts of an unknown phospholipid and two unidentified spots inferring the presence of two aminophospholipids. The presence of these unidentified polar lipids could also be used to distinguish strain HO3-R19^T from related genera. The G + C content of the genomic DNA was 65.0 mol%, a value much higher than for *Silanimonas lenta* (50.7 mol%) and *Xylella fastidiosa* (about 51–53 mol%) but lower than for *A. voraii* (75 mol%), *P. broegbernensis* (70.1 mol%), *L. enzymogenes* (69–70 mol%) and *T. haemolytica* (68.5 mol%). Characteristics that differentiate strain HO3-R19^T from phylogenetically related species are given in Table 1; other detailed characteristics determined are given in the species description below.

In conclusion, the 16S rRNA gene sequence, physiological characteristics, fatty acid profile and DNA G + C content of strain HO3-R19^T were quite different from those of its phylogenetic neighbours. In addition, the closest similarity (93.5 %) of the 16S rRNA gene sequence of strain HO3-R19^T with a recognized bacterium (the type strain of *P.*

suwonensis) was much lower than the threshold level that is generally used to define a new genus (Ludwig *et al.*, 1998). Therefore, we propose that isolate HO3-R19^T should be classified as representing a novel species in a new genus, *Arenimonas donghaensis* gen. nov., sp. nov., within the family *Xanthomonadaceae*, class *Gammaproteobacteria*.

Description of *Arenimonas* gen. nov.

Arenimonas (A.re'ni.mo.nas. L. fem. n. *arena* sand; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Arenimonas* a sand monad, referring to a bacterium isolated from sand).

Cells are aerobic, Gram-negative, non-spore-forming rods. Oxidase- and catalase-positive. Nitrate and nitrite are not reduced. Major isoprenoid quinone is Q-8. Predominant cellular fatty acids are iso-branched, such as iso-C_{16:0}, iso-C_{15:0} and iso-C_{17:1}ω9c. Phylogenetically, the genus belongs to the family *Xanthomonadaceae* within the class *Gammaproteobacteria*. The type species is *Arenimonas donghaensis*.

Description of *Arenimonas donghaensis* sp. nov.

Arenimonas donghaensis (dong.ha.en'sis. N.L. fem. adj. *donghaensis* pertaining to Donghae, the Korean name of the East Sea of Korea, where the type strain was isolated).

Cells are straight or slightly curved rods that are 0.4–0.6 µm wide and 1.3–3.0 µm long. Colonies are yellowish white, translucent and convex on R2A agar after 3 days cultivation. Motile by means of a single polar flagellum. The pH range for growth is 7.0–9.0, with an optimum at pH 8.0. The temperature range for growth is 4–37 °C, with an optimum at 28 °C. Growth occurs at NaCl concentrations of 0–3 % (w/v) (optimum of 1–2 %). Does not grow under anaerobic conditions. Positive for hydrolysis of casein, tyrosine, DNA and gelatin. Negative for indole production, Voges–Proskauer test, phenylalanine deamination and hydrolysis of starch, urea and cellulose. In API 20NE tests, cells are positive only for hydrolysis of gelatin, and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, β-galactosidase and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In API ZYM tests, cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetylglucosaminidase, α-mannosidase and α-fucosidase. Predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, a small amount of an unknown phospholipid and two unknown aminophospholipids. Major fatty acids are iso-C_{16:0},

Table 1. Characteristics used to distinguish between strain HO3-R19^T and other closely related bacteria

Taxa: 1, strain HO3-R19^T; 2, *Thermomonas haemolytica*; 3, *Luteimonas mephitis*; 4, *Pseudoxanthomonas broegbernensis*; 5, *Stenotrophomonas maltophilia*; 6, *Xylella fastidiosa*; 7, *Xanthomonas campestris*; 8, *Lysobacter enzymogenes*; 9, *Silanimonas lenta*; 10, *Aquimonas voraii*. Data for reference taxa were taken from Busse *et al.* (2002), Chen *et al.* (2002), Tóth *et al.* (2001), Finkmann *et al.* (2000), Vauterin *et al.* (1995, 1996), Palleroni & Bradbury (1993), Wells *et al.* (1987), Palleroni (1984), Christensen & Cook (1978), Sullivan *et al.* (2003), Lee *et al.* (2005) and Saha *et al.* (2005). NA, No data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	+	+	NA	+	+	+	+	—	+	+
Temperature optimum (°C)	28	37–50	28	28	35	26–28	28	25–35	47	30
Catalase	+	+	NA	+	+	+	+	+	+	+
Oxidase	+	+	NA	+	+	—	—	+	+	+
Nitrate reduction	—	—	—	—	+	—	—	—	—	—
Aesculin hydrolysis	—	—	—	—	+	—	+	+	—	—
Susceptibility* to:										
Ampicillin (10 µg)	—	+	—	—	—	+	—	NA	+	NA
Penicillin G (10 IU)	—	+	—	—	—	—	—	—	+	NA
Erythromycin (30 µg)	+	+	—	—	—	NA	—	NA	+	NA
Kanamycin (30 µg)	+	+	V+	—	+	+	+	NA	+	NA
Neomycin (30 µg)	+	+	V—	+	—	NA	—	NA	+	NA
Streptomycin (10 µg)	+	+	—	—	—	—	+	—	+	NA
Major fatty acids	iso-C _{16:0} , iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{16:0}	iso-C _{15:0} , iso-C _{17:1} , iso-C _{17:0}	iso-C _{15:0} , anteiso-C _{15:0} , C _{16:0}	iso-C _{15:0} , anteiso-C _{15:0}	C _{16:0} , C _{16:1} , C _{17:0}	iso-C _{15:0} , C _{16:1} , iso-C _{17:1}	iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{16:0}	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{16:0}
Major hydroxy fatty acids	iso-C _{11:0} 3-OH	iso-C _{11:0} 3-OH	iso-C _{11:0} 3-OH	C _{16:0} 2-OH	C _{13:0} iso 3-OH	C _{10:0} 2-OH	iso-C _{13:0} 3-OH	iso-C _{11:0} 3-OH	iso-C _{11:0} 3-OH	iso-C _{11:0} 3-OH
Major polar lipids†	DPG, PE, PG, PL, APL	DPG, PE, PG	NA	NA	DPG, PE, PG	NA	DPG, PE, PG, PME, PL	NA	DPG, PE, PG, PL	NA
DNA G + C content (mol%)	65.0	68.5	NA	70.1	62–66	51–53	65–66	69–70	50.7	75

*V+, Most strains not susceptible; V—, most strains susceptible.

†APL, Unknown aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid; PME, phosphatidylmethylethanolamine.

iso-C_{15:0} and iso-C_{17:1ω9c} when grown on R2A for 3 days. The DNA G + C content is 65.0 mol% (HPLC).

The type strain, HO3-R19^T (=KACC 11381^T=DSM 18148^T), was isolated from seashore sand in Pohang, Korea.

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