Algoriphagus namhaensis sp. nov., isolated from seawater

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A Gram-staining-negative, non-motile, non-spore-forming bacterial strain, DPG-3^T, was isolated from seawater from the South Sea in Korea, and its taxonomic position was investigated using a polyphasic approach. Strain DPG-3^T grew optimally at 30 °C and in the presence of 2 % (w/v) NaCl. In a neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DPG-3^T fell within a clade comprising *Algoriphagus* species and appeared most closely related to *Algoriphagus halophilus* JC 2051^T (96.1 % 16S rRNA gene sequence similarity) and *Algoriphagus lutimaris* S1-3^T (96.4 %). The type strains of other *Algoriphagus* species showed 16S rRNA gene sequence similarities of 92.9–96.0 % with strain DPG-3^T. The predominant menaquinone of strain DPG-3^T was MK-7. The major fatty acids were iso-C_{15:0} and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (summed feature 3). The major polar lipids detected in strain DPG-3^T were phosphatidylcholine, phosphatidylethanolamine and an unidentified lipid. The genomic DNA G + C content was 44.8 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain DPG-3^T is considered to represent a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus namhaensis* sp. nov. is proposed. The type strain is DPG-3^T (=KCTC 23419^T=CCUG 60523^T).

The genus *Algoriphagus*, which was first proposed by Bowman *et al.* (2003), belongs to the phylum *Bacteroidetes* (Nedashkovskaya *et al.*, 2007). At the time of writing, this genus comprises at least 19 recognized species (Euzéby, 1997, 2010), including the recently described species *Algoriphagus aquatilis* (Liu *et al.*, 2009), *Algoriphagus olei* (Young *et al.*, 2009) and *Algoriphagus lutimaris* (Park *et al.*, 2010). Members of the genus *Algoriphagus* have been isolated from sea ice, seawater, algae, marine sediments, a marine solar saltern, soils and freshwater (Nedashkovskaya *et al.*, 2007; Yoon *et al.*, 2005a, b, 2006; Copa-Patiño *et al.*, 2008; Liu *et al.*, 2009; Young *et al.*, 2009). In this study, a bacterial strain is described, designated DPG-3^T, which was isolated from seawater collected near Geojedo, Korea, from the South Sea. Comparative 16S rRNA gene sequence analysis indicated that strain DPG-3^T was phylogenetically affiliated to the genus *Algoriphagus*. The aim of the present work was to determine the exact taxonomic position of strain DPG-3^T by using a polyphasic approach that included the determination of chemotaxonomic and pheno-

typic properties and detailed phylogenetic investigations based on 16S rRNA gene sequences.

Strain DPG-3^T was isolated, by the standard dilution plating technique, on marine agar 2216 (MA; Difco) at 25 °C. Cell biomass of strain DPG-3^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 3 days in marine broth 2216 (MB; Difco) at 30 °C. Cell mass for the analysis of the fatty acids of this strain was harvested from MA plates after incubation for 3 days at 30 °C. Cell biomass of *Algoriphagus ratkowskyi* CIP 107452^T (the type strain of the type species of the genus *Algoriphagus*), which was used as a reference strain for polar lipid analysis, was obtained from cultures grown for 3 days in MB at 30 °C. The morphological, physiological and biochemical characteristics of strain DPG-3^T were investigated using cells grown on MA at 30 °C. Cell morphology was examined under a light microscope (E600; Nikon) and a transmission electron microscope (CM-20; Philips). Transmission electron microscopy was also used to determine the position and number of flagella on cells from an exponentially growing culture. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gliding motility

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DPG-3^T is HQ401024.

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

was investigated as described by Bowman (2000). Gram staining was determined by using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Growth at 30 °C in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated by using trypticase soy broth, which was prepared according to the formula of the Difco medium except that NaCl was excluded and either 0.45 % (w/v) $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ or 0.06 % (w/v) KCl was added. Growth at 30 °C with various NaCl concentrations (2.0–10.0 %, w/v, in increments of 1.0 %) was also investigated in MB. Growth at 4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C was measured on MA. The pH range for growth, at 30 °C, was determined in MB that had been adjusted to pH 4.5–9.5 (in increments of 0.5 pH unit) by the addition of HCl or Na_2CO_3 ; pH values were verified after autoclaving. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber (Thermo Fisher Scientific) on MA and on MA supplemented with potassium nitrate (0.1 %, w/v); in both cases, the medium was prepared anaerobically, under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA, with the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80 and urea were investigated as described previously (Lányi, 1987) except that artificial seawater was used for the preparation of the media. The artificial seawater contained per litre distilled water: 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Bruns *et al.*, 2001). H_2S production was tested as described by Bruns *et al.* (2001). The presence of flexirubin-type pigments was investigated by the KOH test, as described by Reichenbach (1992). For pigment-absorption spectrum analysis, strain DPG-3^T was cultivated in MB aerobically in the dark at 30 °C. The cells in the resultant culture were collected by centrifugation, washed twice in MOPS buffer (0.01 M MOPS/NaOH, 0.1 M KCl and 0.001 M MgCl_2 ; pH 7.5) and disrupted by sonication with a Sonifier 450 (Branson). After removal of the cell debris by centrifugation, the absorption spectrum of the supernatant solution was examined in a DU800 spectrophotometer (Beckman Coulter). Acid production from carbohydrates was determined as described by Leifson (1963). Susceptibility to antibiotics was tested on MA plates by using antibiotic discs containing ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (100 µg), gentamicin (30 µg), kanamycin (30 µg), lincomycin (15 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin (15 µg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 µg) or tetracycline (30 µg). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strain DPG-3^T are given in the species description (see below) or in Table 1. The sonicated cell extracts of strain DPG-3^T showed an absorption peak maximum at 480 nm, indicating the

Table 1. Differential phenotypic characteristics of strain DPG-3^T and the type strains of *A. halophilus* and *A. lutimaris*

Strains: 1, DPG-3^T (data from this study); 2, *A. halophilus* JC 2051^T (=KCTC 12051^T; data from this study; Yi & Chun, 2004; Park *et al.*, 2010); 3, *A. lutimaris* S1-3^T (Park *et al.*, 2010). All strains are Gram-staining-negative, rod-shaped, and positive for catalase, oxidase, hydrolysis of gelatin, acid production from D-glucose and maltose, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase activity, and susceptibility to carbenicillin, chloramphenicol, lincomycin and oleandomycin. All strains are negative for flagellation, gliding motility, production of flexirubin-type pigments, nitrate reduction, H_2S and indole production, arginine dihydrolase, hydrolysis of agar and urea, acid production from *myo*-inositol, D-mannitol, D-ribose and D-sorbitol, lipase (C14), α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase activity, and susceptibility to ampicillin, penicillin G, cephalothin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Absorption peak maximum (nm)	480	475	467
NaCl requirement for growth	+	+	–*
Optimal growth temperature (°C)	30	35	30
Hydrolysis of:			
Casein	+	–	+
DNA	–	–	+
Starch	+	–	–
Tyrosine	+	–	–
Acid production from:			
L-Arabinose	–	+	+
Cellobiose	–	+	–
D-Fructose	w	+	–
D-Galactose	–	–	+
Lactose	–	+	+
D-Mannose	–	+	+
Melezitose	–	+	–
Melibiose	–	+	–
Raffinose	–	+	+
L-Rhamnose	–	+	+
Sucrose	–	+	–
Trehalose	–	–	+
D-Xylose	w	+	–
Enzyme activity (API ZYM)			
Esterase (C4)	+	+	–
Valine arylamidase	–	w	–
Cystine arylamidase	–	+	+
β -Galactosidase	–	+	–
α -Glucosidase	–	+	–
β -Glucosidase	–	+	–
Susceptibility to:			
Novobiocin	+	–	–
Tetracycline	–	+	–
DNA G + C (mol%)	44.8	37	41.4

*Growth with 0 % NaCl occurs when Mg^{2+} ions are added.

presence of carotenoids. A negative KOH test indicated that no flexirubin-type pigments were produced.

Chromosomal DNA was isolated and purified as described by Yoon *et al.* (1996), except that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene sequence and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The almost complete 16S rRNA gene sequence of strain DPG-3^T determined in this study comprised 1444 nt (equivalent to about 96 % of the *Escherichia coli* 16S rRNA gene sequence). In a neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain DPG-3^T fell within a clade comprising species of the genus *Algoriphagus* (Fig. 1). In terms of 16S rRNA gene sequences, strain DPG-3^T appeared most closely related to *Algoriphagus halophilus* JC 2051^T (96.1 % sequence similarity) and *Algoriphagus lutimaris* S1-3^T (96.4 %). The type strains of other recognized species of the genus *Algoriphagus* showed <96.0 % 16S rRNA gene sequence similarity with the novel strain.

Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and an ODS-A (250 × 4.6 mm) column (YMC). The predominant isoprenoid quinone found in strain DPG-3^T, menaquinone-7 (MK-7), was the same as that found in recognized members of the genus *Algoriphagus* (Nedashkovskaya *et al.*, 2004, 2007). Fatty acids were saponified, methylated and extracted using version 4.0 of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed on a 6890 gas chromatograph (Hewlett Packard) and identified by using

the TSBA40 database of the Microbial Identification System (Sasser, 1990). The cellular fatty acid profile of strain DPG-3^T (determined in this study) and those of the type strains of *A. halophilus* and *A. lutimaris* (determined using the same methods and conditions) are shown in Table 2. The major fatty acids (each representing >10 % of total fatty acids) detected in strain DPG-3^T were iso-C_{15:0} and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (summed feature 3) (Table 2). The fatty acid profile of strain DPG-3^T was essentially similar to those of recognized species of the genus *Algoriphagus*, although there were differences in the proportions of some fatty acids (Table 2; Young *et al.*, 2009). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with molybdophosphoric acid, molybdenum blue, ninhydrin or α-naphthol (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987) or with Dragendorff's reagent (Sigma). Although the polar lipid profile of strain DPG-3^T was generally similar to that of *A. ratkowskyi* CIP 107452^T, with phosphatidylcholine, phosphatidylethanolamine and an unidentified lipid identified as the major polar lipids in both of these strains, minor amounts of an unidentified phospholipid and an additional unidentified lipid were only detected in *A. ratkowskyi* CIP 107452^T (Supplementary Fig. S1, available in IJSEM Online). The genomic DNA G+C content of strain DPG-3^T, determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC, was 44.8 mol%. The results of the chemotaxonomic analyses supported those of the phylogenetic analysis, indicating that strain DPG-3^T had several properties in common with established species of the genus *Algoriphagus* (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004, 2007).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between *Algoriphagus namhaensis* DPG-3^T and related species. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Filled circles indicate nodes that were also recovered in the trees generated using both maximum-likelihood and maximum-parsimony algorithms. *Cytophaga hutchinsonii* ATCC 33406^T (M58768) was used as an outgroup. Bar, 0.01 substitution per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain DPG-3^T and the type strains of *A. halophilus* and *A. lutimaris*

Strains: 1, DPG-3^T (data from this study); 2, *A. halophilus* KCTC 12051^T (Park *et al.*, 2010); 3, *A. lutimaris* S1-3^T (Park *et al.*, 2010). Fatty acids that represented <1.0% in all strains were omitted. —, Not detected or <1.0%.

Fatty acid	1	2	3
Straight-chain			
C _{15:0}	0.6	—	2.2
C _{16:0}	0.1	—	1.1
Branched			
anteiso-C _{11:0}	—	1.9	1.5
iso-C _{15:0}	29.8	25.1	21.3
anteiso-C _{15:0}	3.9	3.2	2.6
iso-C _{16:0}	9.0	5.0	4.6
iso-C _{16:1}	9.6	3.2	1.2
iso-C _{17:1} ω9c	3.7	10.3	9.4
Unsaturated			
C _{16:1} ω5c	1.3	3.5	3.9
C _{17:1} ω6c	2.1	1.8	3.2
Hydroxy			
C _{16:0} 3-OH	0.6	—	1.4
iso-C _{15:0} 3-OH	2.7	2.5	2.2
iso-C _{16:0} 3-OH	2.4	2.0	1.4
iso-C _{17:0} 3-OH	7.5	7.1	9.3
Summed features*			
3	15.2	19.4	23.5
4	3.7	3.9	2.7
Unknown			
ECL 13.565†	0.5	2.0	—
ECL 14.959	1.2	—	—

*Summed features represent groups of two or more fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; summed feature 4 comprised iso-C_{17:1} and/or anteiso-C_{17:1}.

†ECL, equivalent chain-length.

Strain DPG-3^T was, however, distinguishable from *A. halophilus* and *A. lutimaris* in terms of several phenotypic characteristics (Table 1; Park *et al.*, 2010). Together, the phylogenetic analysis and phenotypic properties of the novel strain indicate that it is distinct from all established species of the genus *Algoriphagus*, including its closest known relatives, *A. halophilus* and *A. lutimaris* (Stackebrandt & Goebel, 1994). Therefore, on the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain DPG-3^T is considered to represent a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus namhaensis* sp. nov. is proposed.

Description of *Algoriphagus namhaensis* sp. nov.

Algoriphagus namhaensis (nam.ha.en'sis. N.L. masc. adj. *namhaensis* of Namhae, the Korean name for the South Sea in Korea, where the type strain was isolated).

Cells are Gram-staining-negative, non-spore-forming, non-flagellated rods. Most measure 0.2–0.4 × 1.0–5.0 µm, but a few measuring >10 µm in length are also observed. After incubation for 3 days at 30 °C, colonies on MA are circular, convex, smooth, glistening, reddish orange in colour and 0.5–1.0 mm in diameter. Optimal growth occurs at 30 °C; growth occurs at 10 and 40 °C, but not at 4 or 45 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2% (w/v) NaCl; growth occurs with NaCl at 0.5–6.0% (w/v). Mg²⁺ ions are required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine and xanthine are not. The predominant menaquinone is MK-7. The major fatty acids (each >10% of total fatty acids) are iso-C_{15:0} and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (summed feature 3). The major polar lipids are phosphatidylcholine, phosphatidylethanolamine and an unidentified lipid. Other phenotypic properties are shown in Table 1.

The type strain, DPG-3^T (=KCTC 23419^T=CCUG 60523^T), was isolated from seawater collected near Geoje-do, Korea, from the South Sea. The genomic DNA G+C content of strain DPG-3^T is 44.8 mol%.

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