Algoriphagus aquatilis sp. nov., isolated from a freshwater lake

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A Gram-negative, non-spore-forming, non-motile bacterium, strain A8-7^T, was isolated from fresh water of a slightly alkaline lake, Longhu Lake, in Daqing, north-east China, and its taxonomic position was studied by using a polyphasic approach. Strain A8-7^T was aerobic, heterotrophic and positive for catalase and oxidase. It grew at 20–37 °C (optimum 30 °C) and pH 5.5–10.5 (optimum pH 7.5) and in the presence of 0–3 % (w/v) NaCl. It formed pink-pigmented, smooth and circular colonies, 1–2 mm in diameter, on R3A-V agar plates after incubation at 30 °C for 3 days. Cells of strain A8-7^T were rods, 0.2–0.4 μm wide and 1.6–4.0 μm long. The major fatty acids (>10 %) were iso-$C_{15:0}$ (40.3 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 12.1 %). The menaquinone was MK-7. The DNA G+C content was 43 mol% (T_m). Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain A8-7^T was phylogenetically related to members of the genus *Algoriphagus*, with sequence similarities of 92.6–95.2 %, the highest sequence similarity being to the sequence from *Algoriphagus mannitolivorans* IMSNU 14012^T. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain A8-7^T was considered to represent a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus aquatilis* sp. nov. is proposed. The type strain is A8-7^T (=CGMCC 1.7030^T =NBRC 104237^T).

The genus *Algoriphagus* was proposed by Bowman *et al.* (2003) for heterotrophic, Gram-negative, non-motile, strictly aerobic, pink-pigmented and rod-shaped bacteria, and was initially described as accommodating cold-adapted micro-organisms because strains of the type species, *Algoriphagus ratkowskyi*, were isolated from sea ice and from saline lake cyanobacterial mats collected in Antarctica (Bowman *et al.*, 2003). Later, mesophilic *Algoriphagus* species were isolated and the description of the genus was emended (Nedashkovskaya *et al.*, 2004, 2007). Members of the genus *Algoriphagus* form a phylogenetic cluster with the genera *Hongiella* (Yi & Chun, 2004) and *Chimaereicella* (Tiago *et al.*, 2006). Based on phylogenetic evidence and chemotaxonomic and phenotypic data, the genera *Hongiella* and *Chimaereicella* were combined with the genus *Algoriphagus* by Nedashkovskaya *et al.* (2007). At the time of writing, the genus *Algoriphagus* comprises 16 species with validly published names: *Algoriphagus ratkowskyi* (the type species; Bowman *et al.*, 2003), *Algoriphagus aquimarinus*, *Algoriphagus chordae* and *Algoriphagus winogradskyi* (Nedashkovskaya *et al.*, 2004), *Algoriphagus halophilus* (Yi & Chun, 2004; Nedashkovskaya *et al.*, 2004), *Algoriphagus antarcticus* (Van Trappen *et al.*, 2004), *Algoriphagus yeomjeoni* (Yoon *et al.*, 2005a), *Algoriphagus locisalis* (Yoon *et al.*, 2005b), *Algoriphagus* (*Hongiella*) *mannitolivorans* and *Algoriphagus* (*Hongiella*) *ornithinivorans* (Yi & Chun, 2004), *Algoriphagus* (*Hongiella*) *marincola* (Yoon *et al.*, 2004), *Algoriphagus* (*Chimaereicella*) *alkaliphilus* (Tiago *et al.*, 2006), *Algoriphagus* (*Chimaereicella*) *boritolerans* (Ahmed *et al.*, 2007), *Algoriphagus terrigena* (Yoon *et al.*, 2006), *Algoriphagus vanfongensis* (Nedashkovskaya *et al.*, 2007) and *Algoriphagus hitonicola* (Copa-Patiño *et al.*, 2008). In this study, we report the isolation and taxonomic characterization of an *Algoriphagus*-like bacterium, strain A8-7^T, which was isolated from surface water of a slightly alkaline lake, Longhu Lake, in Daqing, north-east China (46° 43′ N 124° 24′ E).

Longhu Lake has the following characteristics: depth, 3.0 m; pH 8.9; 23 °C; chemical oxygen demand, 25 mg

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of A8-7^T is EU313811.

Maximum-parsimony and maximum-likelihood trees based on 16S rRNA gene sequences and a table of cellular fatty acid contents of strain A8-7^T and strains of other *Algoriphagus* species are available as supplementary material with the online version of this paper.

l^{-1}; biological oxygen demand, 15 mg l^{-1}; total nitrogen, 1.1 mg l^{-1}; total phosphorus, 0.10 mg l^{-1}. For isolation, a serial dilution of a surface-water sample was spread on low-organic Luria–Bertani agar plates (l^{-1}: 1.0 g tryptone (Difco), 0.5 g yeast extract (Difco) and 10.0 g NaCl; pH 8.0) and incubated at 30 °C for 2 days. Subcultures of strain A8-7^T were performed using R3A-V medium (Tiago *et al.*, 2006) or YP medium (containing l^{-1}: 3.0 g tryptone, 3.0 g yeast extract, 0.5 g $MgSO_4 . 7H_2O$, 0.3 g NaCl). Strain A8-7^T cannot grow in marine 2216 medium (Difco).

Routine cultivation was conducted at 30 °C with R3A-V agar. Motility and morphology were observed using phase-contrast microscopy and scanning electron microscopy (Quanta 200; FEI). Flagellation was examined with tannin staining according to Dong & Cai (2001) and transmission electron microscopy (H600; Hitachi). The Gram reaction was determined by Gerhardt *et al.* (1994).

Catalase and oxidase activities and hydrolysis of casein, gelatin, aesculin, L-tyrosine, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). H_2S production was determined by Bruns *et al.* (2001). Growth under anaerobic conditions was determined by incubation in an anaerobic chamber with anaerobically prepared YP broth. Growth at various NaCl concentrations (0–3.5 %, in increments of 0.5 %, w/v) was investigated in R3A-V broth. Growth at various temperatures was tested using R3A-V broth and agar at pH 8 by incubation at various temperatures (16, 20, 25, 30, 35, 37 and 40 °C). The pH range for growth was determined in YP broth adjusted to pH 5.0–11.0, in increments of 0.5 pH units, using 1 M NaOH or 1 M HCl.

The presence of flexirubin-like pigments was tested according to Fautz & Reichenbach (1980). Extracellular glycans were identified with the Congo red absorption test (McCammon & Bowman, 2000). *In vivo* pigment absorption spectrum analysis was examined as described by Yoon *et al.* (2004) using a Unico UV-2802H spectrophotometer (Shanghai Optical Company).

Carbon source assimilation was determined using API 50 CH test strips (bioMérieux), using 0.1 M Tris/HCl buffer, pH 8.0, supplemented with API AUX medium (bioMérieux). Acid production from various substrates was determined using API 50 CH test strips and 50 CHB/E medium (bioMérieux) according to the manufacturer's instructions. Additional biochemical properties and enzyme activities were detected with the API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Susceptibility to antibiotics was determined on R3A-V agar plates using filter-paper discs (Beijing Pharmaceutical Company) containing various antibiotics, specified in the species description. All above tests were performed in triplicate.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene were carried out according to the procedures given by Kim *et al.* (1998). The almost-complete nucleotide sequence of the 16S rRNA gene (1484 nt) was compared with available 16S rRNA gene sequences in the GenBank database using the BLAST program (Altschul *et al.*, 1990) at NCBI (http://www.ncbi.nlm.nih.gov). Multiple alignment with sequences of close relatives was performed by using the CLUSTAL X program (Thompson *et al.*, 1994) and phylogenetic analyses were then carried out using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. A phylogenetic tree was reconstructed with the neighbour-joining method from the calculated evolutionary distances. Phylogenetic analyses based on the 16S rRNA gene revealed that strain A8-7^T was phylogenetically related to members of the genus *Algoriphagus*, with sequence similarities of 92.6–95.2 %, the highest similarity being shown to the sequence from *A. mannitolivorans* IMSNU 14012^T. Strain A8-7^T, together with *A. mannitolivorans* IMSNU 14012^T, formed a distinct phylogenetic lineage within the genus *Algoriphagus* (Fig. 1), indicating that it was likely to represent a novel species of the genus *Algoriphagus*. This phylogenetic placement was also found in trees constructed using the maximum-parsimony and

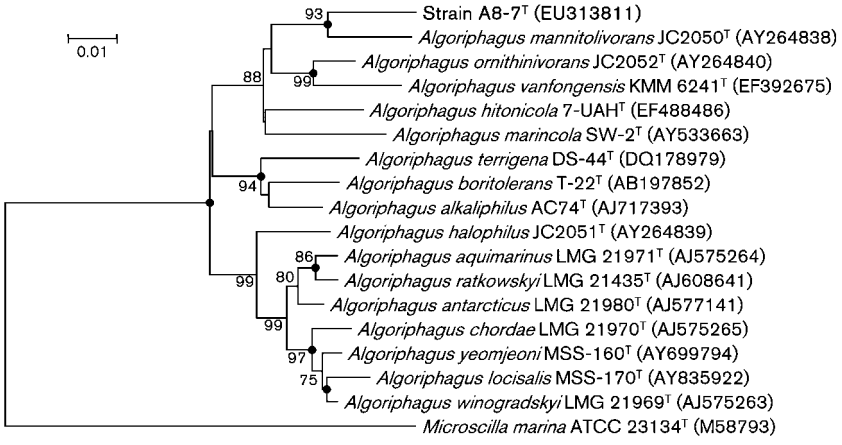

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain A8-7^T and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >70 % are shown at branch points. Filled circles indicate branches that were also recovered using both maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. The sequence of *Microscilla marina* ATCC 23134^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential properties of strain A8-7^T and the type strains of other *Algoriphagus* species

Strains: 1, *Algoriphagus aquatilis* sp. nov. A8-7^T; 2, *A. mannitolivorans* JC2050^T (data from Yi & Chun, 2004); 3, *A. alkaliphilus* AC74^T (Tiago *et al.*, 2006); 4, *A. boritolerans* T-22^T (Ahmed *et al.*, 2007); 5, *A. ornithinivorans* JC2052^T (Yi & Chun, 2004); 6, *A. halophilus* JC2051^T (Yi & Chun, 2004); 7, *A. terrigena* DS-44^T (Yoon *et al.*, 2006); 8, *A. marincola* SW-2^T (Yoon *et al.*, 2004); 9, *A. hitonicola* 7-UAH^T (Copa-Patiño *et al.*, 2008); 10, *A. vanfongensis* KMM 6241^T (Nedashkovskaya *et al.*, 2007); 11, *A. aquimarinus* KMM 3958^T (Nedashkovskaya *et al.*, 2004); 12, *A. winogradskyi* KMM 3956^T (Nedashkovskaya *et al.*, 2004); 13, *A. ratkowskyi* IC025^T (Bowman *et al.*, 2003); 14, *A. antarcticus* LMG 21980^T (Van Trappen *et al.*, 2004); 15, *A. locisalis* MSS-170^T (Yoon *et al.*, 2005b); 16, *A. yeomjeoni* MSS-160^T (Yoon *et al.*, 2005a); 17, *A. chordae* KMM 3957^T (Nedashkovskaya *et al.*, 2004). All strains are positive for catalase, alkaline phosphatase and aesculin hydrolysis. +, Positive; −, negative; W, weakly positive; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Nitrate reduction	−	+	+	−	−	−	+	V	+	−	−	+	V	−	−	−	−
NaCl for growth (%)	0–3	0–7	0–3	0–3	0–10	0–8	1–7	1–9	1.5–5	0–8	0–10	0–6	0–6	0–5	1–9	1–9	1–10
Hydrolysis of:																	
Casein	W	−	−	ND	−	−	+	+	ND	−	+	−	+	−	−	+	−
Gelatin	−	+	+	−	+	+	−	−	ND	+	+	+	V	−	−	V	−
Starch	+	+	+	+	+	+	−	+	+	−	−	+	V	−	−	−	−
DNA	−	+	+	ND	+	−	ND	+	+	−	+	−	V	−	ND	−	−
Tween 20	+	−	ND	ND	−	+	+	+	ND	−	+	+	+	ND	+	+	+
Tween 40	+	−	ND	ND	+	+	+	+	ND	−	+	+	+	ND	+	+	+
Tween 80	+	−	ND	ND	+	+	+	+	−	−	+	−	−	ND	+	+	−
H_2S production	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Oxidase, β-galactosidase	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Acid production from:																	
L-Arabinose	−	−	+	−	−	−	+	+	−	−	−	−	+	−	+	−	−
D-Fructose	W	−	+	−	−	ND	+	V	+	−	ND	ND	+	−	+	+	ND
D-Glucose	W	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Lactose	W	−	+	+	−	−	+	+	+	−	+	+	−	−	+	+	+
Maltose	W	−	+	+	−	+	+	+	+	+	−	+	+	−	+	+	+
D-Mannose	W	−	+	+	−	ND	+	+	+	+	+	+	+	−	+	−	+
Melibiose	W	−	+	−	−	−	+	+	+	−	+	+	+	−	+	+	+
L-Rhamnose	−	−	−	+	−	−	−	−	−	+	+	+	+	−	+	+	+
Sucrose	W	+	+	−	−	+	+	+	+	−	+	+	−	−	+	+	+
D- and L-Xylose	−	−	−	−	−	+	+	+	−	+	+	+	+	−	+	+	+
N-Acetyl-D-glucosamine	−	−	−	+	−	+	ND	−	+	+	+	+	+	−	ND	−	−
Utilization of:																	
L-Arabinose	−	+	+	−	−	+	+	+	ND	+	+	+	+	−	+	−	+
D-Glucose, maltose, D-mannose	+	+	+	+	+	+	+	+	ND	+	+	+	+	−	+	+	+
D-Mannitol	−	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−
Sorbitol	−	−	+	−	−	−	ND	−	ND	−	−	−	+	ND	ND	ND	−
N-Acetyl-D-glucosamine	+	+	−	−	+	+	ND	−	ND	+	+	+	+	−	ND	−	+
DNA G+C content (mol%)	43	42	43.5	42.5	38	37	49	43	43.4	43.8	41	39	35	40.6	42	41	40

maximum-likelihood methods (Supplementary Fig. S1, available in IJSEM Online).

The G+C content of the DNA was determined by thermal denaturation (Marmur & Doty, 1962) and DNA from *Escherichia coli* K-12 was used as a control. The DNA G+C content of strain A8-7^T was 43 mol% (T_m), which is within the range reported for the genus *Algoriphagus* (35–44 mol%; Nedashkovskaya *et al.*, 2007).

Cells of strain A8-7^T were non-motile, non-spore-forming rods, 0.2–0.4 μm wide and 1.6–4.0 μm long. Colonies on R3A-V or YP medium were pink, smooth, circular, convex and 1–2 mm in diameter after incubation for 3 days. Colony colour was variable and dependent on the pH. Faint pink pigmentation was found after growth on alkaline medium (>pH 8). The absorption maximum of crude extracts was 485 nm, which is similar to other members of the genus *Algoriphagus* (Copa-Patiño *et al.*, 2008; Yi & Chun, 2004; Yoon *et al.*, 2004).

Cellular fatty acid content was determined by the MIDI Sherlock Microbial Identification System (Microbial ID) with cells grown on R3A-V plates at 30 °C for 3 days, according to the manufacturer's instructions. Strain A8-7^T had a fatty acid profile similar to those of closely related *Algoriphagus* species (Supplementary Table S1). The dominant fatty acids were iso-$C_{15:0}$ (40.3 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$2-OH; 12.1 %), which are also the dominant fatty acids for other members of the

genus *Algoriphagus*. Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987). Strain A8-7^T contained MK-7 as the sole respiratory menaquinone, as for other members of the genus *Algoriphagus*.

Some phenotypic properties of strain A8-7^T, such as its being strictly aerobic and heterotrophic, the production of non-diffusible pigments and the presence of oxidase and catalase activities, in addition to its chemotaxonomic characteristics, suggested that it should be placed in the genus *Algoriphagus*. However, several properties, including hydrolysis of gelatin, starch, DNA and Tweens 20, 40 and 80, H_2S production, acid production from several carbohydrates and the utilization of several carbon sources, differentiated strain A8-7^T from other species of the genus *Algoriphagus* (Table 1).

On the basis of the evidence described above, strain A8-7^T is considered to represent a novel species of the genus *Algoriphagus*, and the name *Algoriphagus aquatilis* sp. nov. is proposed.

Description of *Algoriphagus aquatilis* sp. nov.

Algoriphagus aquatilis (a.qua′ti.lis. L. masc. adj. *aquatilis* aquatic, pertaining to the isolation of the type strain from fresh water).

Exhibits the following properties in addition to those given in Table 1. Cells are Gram-negative, non-motile, non-spore-forming rods, 0.2–0.4 μm wide and 1.6–4.0 μm long. Positive for catalase. Strictly aerobic and heterotrophic. Growth occurs at 20–37 °C (optimum 30 °C) and pH 5.5–10.5 (optimum pH 7.5) and in the presence of 0–3 % (w/v) NaCl (optimum 0.5–1 %). Flexirubin-type pigments and glycans are absent. Negative for urease and arginine dihydrolase. Indole is not produced. Hydrolyses L-tyrosine and Tween 60. With API ZYM tests, strong activity is observed for leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, weak activity for esterase (C4), esterase lipase (C8), cystine arylamidase, β-galactosidase and β-mannosidase, but no activity for lipase (C14), β-glucuronidase and α-fucosidase. Produces acid weakly from D-galactose, methyl α-D-glucoside, aesculin, salicin, cellobiose, trehalose, raffinose, starch, gentiobiose and turanose, but not from glycerol, erythritol, D-ribose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannoside, amygdalin, arbutin, melezitose, glycogen, xylitol, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Utilizes methyl α-D-glucoside, amygdalin, cellobiose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose and turanose, weakly utilizes erythritol, D-adonitol, D-fructose, dulcitol, arbutin and salicin, but does not utilize glycerol, D- or L-arabinose, D-ribose, D- or L-xylose, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, inositol, methyl α-D-mannoside, inulin, xylitol, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. The major fatty acids are iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The major menaquinone is MK-7. Antibiotic sensitivity results as follows (μg per disc unless otherwise stated): resistant to vancomycin (30); weakly resistant to ampicillin (10) and penicillin (10 IU); susceptible to gentamicin (10), carbenicillin (100), polymixin B (300 IU), streptomycin (10), tetracycline (30), kanamycin (30), erythromycin (15), novobiocin (5), chloramphenicol (30), ciprofloxacin (5), norfloxacin (10) and rifampicin (5). The DNA G+C content of the type strain is 43 mol% (T_m).

The type strain is A8-7^T (=CGMCC 1.7030^T =NBRC 104237^T), isolated from fresh water of the slightly alkaline Longhu Lake, Daqing city, north-east China.

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