

Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from sea water and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman *et al.* 2003 and *Hongiella* Yi and Chun 2004

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Four marine heterotrophic, aerobic, pink-pigmented and non-motile bacterial strains were isolated from sea water and algae collected in the Sea of Japan. In a polyphasic taxonomic study, 16S rRNA gene sequence analysis indicated that the strains were phylogenetically highly related to *Algoriphagus ratkowskyi* LMG 21435^T, a member of the phylum *Bacteroidetes*. Further phenotypic, chemotaxonomic and genomic analyses revealed that the strains represent three novel species of the genus *Algoriphagus*, for which the following names are proposed: *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., with type strains KMM 3958^T (=LMG 21971^T=CCUG 47101^T), KMM 3957^T (=LMG 21970^T=CCUG 47095^T) and KMM 3956^T (=LMG 21969^T=CCUG 47094^T), respectively. The species *Hongiella halophila* Yi and Chun 2004 is transferred to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. because of its close phylogenetic relatedness to *Algoriphagus* species and analogous phenotypic and chemotaxonomic properties. The above-mentioned novel species descriptions and species reclassification justify emended descriptions of the genera *Algoriphagus* and *Hongiella*.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 3956^T, KMM 3958^T, KMM 3957^T, LMG 13164^T and LMG 21435^T are AJ575263, AJ575264, AJ575265, AJ575266 and AJ608641, respectively.

A scanning electron micrograph of cells of strain KMM 3956^T is available as supplementary material in IJSEM Online.

The genus *Algoriphagus* was proposed by Bowman *et al.* (2003) for heterotrophic, Gram-negative, non-motile, strictly aerobic, saccharolytic, rod-like, pink-pigmented and cold-adapted strains isolated from sea ice and from saline lake cyanobacterial mats collected in Antarctica. The genus is phylogenetically distantly related to the genus *Cyclobacterium* of the phylum *Bacteroidetes* and was represented by a single species *Algoriphagus ratkowskyi* with type strain IC025^T. A novel genus, *Hongiella*, closely related to the genus *Algoriphagus*, was recognized by Yi & Chun (2004) and comprised three species of pink–orange-pigmented, aerobic, non-motile, heterotrophic and mesophilic bacteria from marine sediments of the Sea of Japan. In the present paper, the taxonomic position of four novel bacteria was studied, which were obtained from sea water and algae from the Sea of Japan and which were related to the genera *Algoriphagus* and *Hongiella*.

Strains KMM 3956^T, KMM 3977 and KMM 3957^T were isolated in Troitsa Bay (Gulf of Peter the Great, Sea of Japan), the former two from the green alga *Acrosiphonia sonderi* and the latter from the brown alga *Chorda filum*. KMM 3958^T was isolated in Amursky Bay (Gulf of Peter the Great) from a sea-water sample. All samples were collected in June 2000. For isolation, 0.1 ml algal homogenates or 0.1 ml sea water was transferred on marine agar 2216 (Difco) at 28 °C, and the isolates were purified and cultivated for further experiments under the same conditions and stored at –80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

Gram-staining reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed by scanning electron microscopy according to the method described by Bruns *et al.* (2001). Strains isolated in this study were Gram-negative, chemo-organotrophic, aerobic, non-motile organisms with short rod-shaped cells, 0.4–0.6 µm in diameter and 1.2–1.5 µm in length (an image of cells of strain KMM 3956^T is available as supplementary material in IJSEM Online). On marine agar, colonies formed by strains KMM 3956^T, KMM 3977 and KMM 3957^T were round, convex, 2–3 mm in diameter, bright-pink coloured and grew into the agar. Colonies of strain KMM 3958^T were a pale-pink colour and grew on the agar surface.

The phylogenetic position of three novel isolates, KMM 3956^T, KMM 3957^T and KMM 3958^T, and the reference strains *A. ratkowskyi* LMG 21435^T and *Cyclobacterium marinum* LMG 13164^T was determined by complete 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the protocol of Niemann *et al.* (1997). 16S rRNA genes were amplified using oligonucleotide primers complementary to highly conserved regions of bacterial 16S rRNA genes. The forward primer was 5'-AGAGTTT-GATCCTGGCTCAG-3' (hybridizing at positions 8–27, according to the *Escherichia coli* numbering system) and the reverse primer was 5'-AAGGAGGTGATCCAGCCGCA-3' (hybridizing at positions 1541–1522). PCR products were purified using a QIAquick PCR purification kit (Qiagen),

according to the manufacturer's instructions. Purified PCR products were sequenced using the ABI Prism Big Dye Terminator cycle sequencing ready reaction kit and an Applied Biosystems 3100 DNA sequencer, using the protocols of the manufacturer (Applied Biosystems). The eight sequencing primers used are listed in Coenye *et al.* (1999). Sequence assembly was performed using the program AUTOASSEMBLER (Applied Biosystems).

The 16S rRNA gene sequences (continuous stretches of 1473 bp for KMM 3956^T, KMM 3957^T and KMM 3958^T, 1436 bp for *A. ratkowskyi* LMG 21435^T and 1476 bp for *C. marinum* LMG 13164^T) and sequences of strains retrieved from EMBL were aligned and a phylogenetic tree was constructed by the neighbour-joining method using the BIONUMERICS software package, version 3.50 (Applied Maths). Unknown bases were discarded from the calculations. Phylogenetic analysis of nearly complete 16S rRNA gene sequences of strains KMM 3956^T, KMM 3957^T and KMM 3958^T revealed sequence similarities of 98.2–98.9 %. The marine isolates represent part of the phylum *Bacteroidetes*. Highest sequence similarities (98.0–99.1 %) were obtained with the sequence of *A. ratkowskyi* LMG 21435^T, determined in the present study. The latter sequence was significantly different from the originally deposited sequence of *A. ratkowskyi* IC025^T (GenBank/EMBL accession no. U85891), in which multiple errors were found (confirmed by J. Bowman, personal communication). Other closest phylogenetic neighbours were the genera *Hongiella*, *Cyclobacterium* and *Belliella* (Yi & Chun, 2004; Raj & Maloy, 1990; Brettar *et al.*, 2004; Fig. 1). Among these and the marine isolates, significantly high sequence similarities were obtained with the species *Hongiella halophila* (96.8–97.5 %), whereas *Hongiella mannitolivorans* and *Hongiella ornithinivorans* were more distantly related (93.7–94.0 and 94.3–94.6 %, respectively). Sequence similarities with the genera *Cyclobacterium* and *Belliella* were in the range 92.8–93.5 %.

DNA G + C contents were determined for the three marine isolates KMM 3956^T, KMM 3957^T and KMM 3958^T, *A. ratkowskyi* LMG 21435^T and *H. halophila* DSM 15292^T. DNA was extracted from strains KMM 3956^T, KMM 3957^T and KMM 3958^T using the protocol described by Marmur (1961) for determination of DNA G + C contents by the thermal denaturation method of Marmur & Doty (1962). DNA base contents of the latter isolates, *A. ratkowskyi* LMG 21435^T and *H. halophila* DSM 15292^T were also determined by HPLC (Mesbah *et al.*, 1989) with DNA extracts prepared using the protocol described by Pitcher *et al.* (1989), modified for large-scale preparation. For the HPLC experiments, the nucleoside mixture was separated by using a Waters SymmetryShield C8 column maintained at a temperature of 37 °C. The solvent was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5 % acetonitrile. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. The DNA G + C contents of the three marine strains KMM 3956^T, KMM 3957^T and KMM

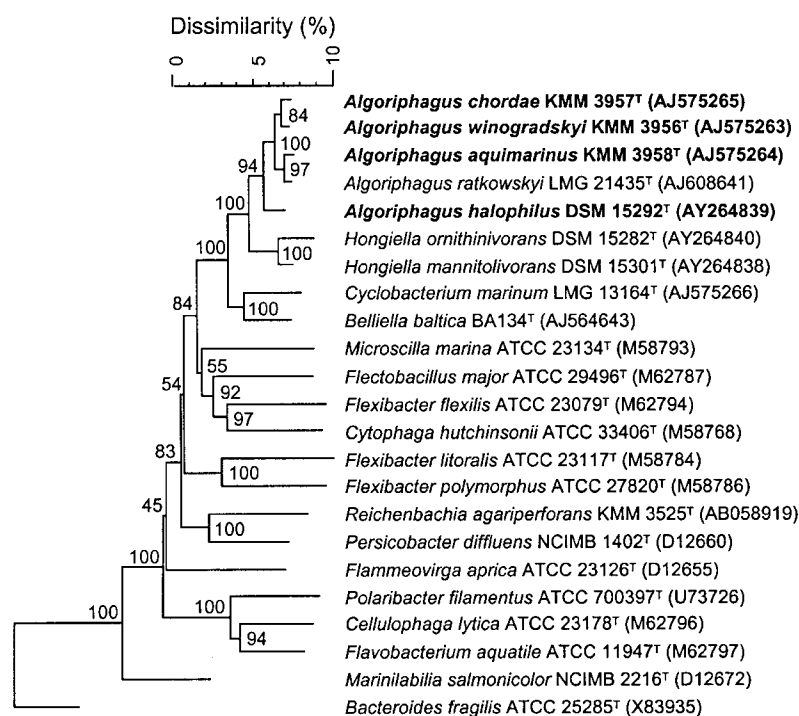


Fig. 1. Distance matrix tree showing the phylogenetic relationships of the genus *Algoriphagus* and representative related taxa, based on 16S rRNA gene sequence comparisons. *Bacteroides fragilis* was used as the outgroup and bootstrap probability values (percentages of 500 tree replications) are indicated at branch points.

3958^T were 42 (T_m) and 39 mol% (HPLC), 40 (T_m) and 37 mol% (HPLC) and 41 (T_m) and 41 mol% (HPLC), respectively. The values for *A. ratkowskyi* and *H. halophila* were 37 mol% (HPLC), similar to the values of 35–36 and 37 mol%, respectively, from the literature (Bowman *et al.*, 2003; Yi & Chun, 2004).

DNA–DNA hybridizations were performed between strains KMM 3956^T, KMM 3977, KMM 3957^T and KMM 3958^T, *A. ratkowskyi* LMG 21435^T and *H. halophila* DSM 15292^T. For hybridizations among strains KMM 3956^T, KMM 3977, KMM 3957^T and KMM 3958^T, DNA was prepared using the protocol of Marmur (1961) and hybridizations were performed spectrophotometrically using the initial renaturation rate method of De Ley *et al.* (1970). For determination of the binding levels between the marine isolates and *A. ratkowskyi* LMG 21435^T and *H. halophila* DSM 15292^T, DNA was prepared using the modified protocol of Pitcher *et al.* (1989) (as described above). For the latter experiments, the microplate method was used as described by Ezaki *et al.* (1989) and Goris *et al.* (1998), using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. Biotinylated DNA was hybridized with unlabelled ssDNA, which was bound non-covalently to microplate wells. Hybridizations were performed at 33 °C in hybridization mixture (2 × SSC, 5 × Denhardt's solution, 2.5 % dextran sulphate, 50 % formamide, 100 µg denatured salmon sperm DNA ml⁻¹, 1250 ng biotinylated probe DNA ml⁻¹). Experiments carried out between strains KMM 3956^T and KMM 3957^T, strains KMM 3956^T and KMM 3958^T and strains KMM 3957^T and KMM 3958^T showed low binding values of 46, 38 and 33 %, respectively; this indicates that the strains represent different species.

DNA–DNA hybridization between KMM 3956^T and KMM 3977 resulted in a binding level of 81 %. Hybridizations performed between *A. ratkowskyi* LMG 21435^T and strains KMM 3956^T, KMM 3957^T and KMM 3958^T revealed binding levels of 14, 18 and 36 %, respectively. Binding levels between *H. halophila* DSM 15292^T and strains KMM 3956^T, KMM 3957^T and KMM 3958^T were 9–13 %. *A. ratkowskyi* LMG 21435^T and *H. halophila* DSM 15292^T had a binding level of 7 %. All these data indicate a separate species status for each of the three marine strains.

Isoprenoid quinones were extracted from lyophilized cells of strains KMM 3956^T, KMM 3957^T and KMM 3958^T and analysed as described by Akagawa-Matsushita *et al.* (1992). Isoprenoid quinone composition was characterized by HPLC (Shimadzu Instruments) using a reverse-phase type Zorbax ODS column (250 × 4.6 mm) and acetonitrile/2-propanol (65:35 v/v) as a mobile phase at a flow rate of 0.5 ml min⁻¹. The column was kept at 40 °C. Menaquinones were detected by monitoring at 270 nm and were identified by comparison with known quinones from reference strain *C. marinum* LMG 13164^T. The major lipoquinone is MK-7.

For fatty acid methyl ester analysis, a loopful of well-grown cells of strains KMM 3956^T, KMM 3957^T and KMM 3958^T, *A. ratkowskyi* LMG 21435^T and the type strains of the three *Hongiella* species were harvested. Fatty acid methyl esters were prepared as described by Vandamme *et al.* (1992) and separated and identified using the Sherlock Microbial Identification System (version 3.0, MIDI Inc.). Predominant cellular fatty acids for all strains analysed were 15:0 iso, 16:0 iso and 17:0 iso 3-OH and summed feature 3

(Table 1). No characteristic fatty acids were observed that clearly distinguished the three marine isolates, the type strain of *A. ratkowskyi* and type strains of species of the genus *Hongiella*. When performing cluster analysis (data not shown), the three marine isolates and the type strains of *A. ratkowskyi* and *H. halophila* group more closely together and separate from *H. mannitolivorans* LMG 22066^T and *H. ornithinivorans* LMG 22068^T owing to significantly higher percentages of summed feature 3 and 16:1 ω 5c (Table 1).

Conventional biochemical tests and carbohydrate fermentation tests were carried out. Spreading growth was observed by cultivation on medium B containing 1 g Bacto peptone l⁻¹ (Difco), 1 g yeast extract l⁻¹ (Difco), 15 g agar l⁻¹ and half-strength natural sea water under high moisture conditions. Gliding motility was determined as described by Bowman (2000). Oxidative or fermentative utilization of glucose was determined on the Hugh & Leifson medium modified for marine bacteria (Lemos *et al.*, 1985).

Degradation of agar, starch, casein, gelatin, cellulose (filter paper and CM-cellulose), chitin, DNA, urea and alginic acids, flexirubin production, growth at different pH, production of acid from carbohydrates and susceptibility to antibiotics were tested as described by Nedashkovskaya *et al.* (2003). Hydrolysis of Tweens 20, 40 and 80, nitrate reduction, production of hydrogen sulphide, acetoin (Voges–Proskauer reaction) and indole, β -galactosidase, oxidase, catalase and alkaline phosphatase activities were tested according to the methods of Gerhardt *et al.* (1994). To study the temperature range for growth, bacteria were cultivated on medium A containing (per litre of an equal volume of natural sea water and distilled water): 5 g Bacto peptone (Difco), 2 g Bacto yeast extract (Difco), 1 g glucose, 0.02 g KH₂PO₄ and 0.05 g MgSO₄·7H₂O. Growth at different NaCl concentrations was checked in medium A prepared in distilled water with 0, 1, 2, 3, 5, 6, 8, 10 and 12 % (w/v) NaCl. Carbon source utilization was tested using commercial API 20NE identification strips following the manufacturer’s instructions, except for the medium, which

Table 1. Fatty acid content (percentage of total) of whole-cell hydrolysates of *Algoriphagus* species and related taxa

Species: 1, *A. ratkowskyi* LMG 21435^T; 2, *A. aquimarinus* sp. nov. KMM 3958^T; 3, *A. chordae* sp. nov. KMM 3957^T; 4, *A. winogradskyi* sp. nov. KMM 3956^T; 5, *A. halophilus* comb. nov. LMG 22067^T; 6, *H. mannitolivorans* LMG 22066^T; 7, *H. ornithinivorans* LMG 22068^T. Only fatty acids comprising more than 1.0 % for one of the strains are indicated. ECL, unidentified fatty acid with equivalent chain-length. —, Not detected.

Fatty acid	1	2	3	4	5	6	7
11:0anteiso	2.1	0.6	1.0	1.2	1.5	0.9	—
14:0iso	1.4	0.7	0.3	0.3	0.6	2.3	0.6
15:0	1.6	1.6	2.5	1.2	1.0	3.0	2.7
15:1 ω 6c	2.1	2.3	1.0	1.3	1.8	4.1	1.1
15:0anteiso	3.6	3.2	1.9	1.6	2.8	3.7	6.1
15:0iso	30.5	38.9	38.1	36.6	28.4	32.7	26.5
15:0iso 3-OH	2.9	1.8	1.6	2.0	2.5	2.7	1.8
15:1isoG	1.1	0.3	—	2.9	—	0.4	0.2
16:0	2.9	1.7	1.4	1.0	—	0.2	0.3
16:1 ω 5c	5.8	5.2	3.6	3.6	3.5	0.6	1.1
16:0 3-OH	1.9	0.5	1.9	0.8	0.7	—	—
16:0iso	3.4	2.4	5.8	3.9	7.7	6.5	12.3
16:0iso 3-OH	2.8	1.0	0.9	1.9	3.1	4.7	3.4
16:1isoH	1.7	2.4	1.5	2.1	3.5	3.5	6.4
17:1 ω 6c	0.5	0.7	1.2	0.8	3.4	7.0	4.5
17:0 2-OH	—	0.3	—	—	0.6	0.4	1.8
17:0iso 3-OH	9.2	5.9	6.4	6.4	5.9	6.4	6.7
17:1anteiso ω 9c	—	—	—	—	—	—	1.0
17:1iso ω 9c	1.5	5.3	4.4	4.0	9.0	6.0	12.2
Summed feature 3*	22.3	20.4	22.2	24.6	19.0	7.4	6.0
Summed feature 4*	0.9	1.8	1.7	1.6	2.7	2.5	2.0
ECL 14:959	—	—	—	—	0.7	1.6	—

*Summed features consist of one or more fatty acids that can not be separated by the Microbial Identification System. Summed feature 3 consisted of 15:0iso 2-OH, 16:1 ω 7c and/or 16:1 ω 7t and summed feature 4 consisted of 17:1isoI and/or 17:1anteisoB.

contained 0.2 g NaNO₃, 0.2 g NH₄Cl, 0.05 g yeast extract (Difco) and 0.4 % (w/v) carbon source per litre of artificial sea water.

Phenotypic characteristics of the strains are given in Table 2 and summarized below in the species descriptions. Strains KMM 3956^T and KMM 3977 were similar to each other and

Table 2. Phenotypic characteristics of *Algoriphagus* strains and related taxa

Species: 1, *A. ratkovskyi*; 2, *A. aquimarinus* sp. nov.; 3, *A. chordae* sp. nov.; 4, *A. winogradskyi* sp. nov.; 5, *A. halophilus* comb. nov.; 6, *H. mannitolivorans*; 7, *H. ornithinivorans*. Data were obtained from Bowman *et al.* (2003), Yi & Chun (2004) and this study. All strains were positive for: respiratory metabolism; oxidase, catalase, β -galactosidase and alkaline phosphatase activities; growth at 10–32 °C and 1–6 % NaCl; hydrolysis of alginate; utilization of L-arabinose, D-glucose, D-lactose, D-mannose and D-sucrose. All strains were negative for: gliding motility; flexirubin pigments; H₂S, indole and acetoin production; hydrolysis of cellulose (filter paper, CM-cellulose) and urea; oxidation of L-sorbose, adonitol, glycerol and mannitol; utilization of citrate; susceptibility to gentamicin, kanamycin, neomycin and polymyxin B. +, Positive; –, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Nitrate reduction	–	–	–	+	–	+	–
NaCl requirement for growth	+	–	+	–	–	–	–
Growth at:							
10 % NaCl	–	+	+	–	–	–	–
37 °C	–	–	–	+	+	+	+
Hydrolysis of:							
Agar	–	+	+	+	–	–	–
Casein	V	+	–	–	–	–	–
Gelatin	–	+	–	+	+	+	+
Starch	V	–	–	+	+	+	+
Chitin	V	–	–	–	–	–	–
DNA	–	+	–	–	–	+	+
Tween 80	–	+	–	–	+	–	+
Acid from:							
L-Arabinose	+	–	–	–	–	–	ND
D-Cellobiose	+	+	+	+	–	+	ND
L-Fucose	–	+	+	–	–	–	ND
D-Galactose	+	–	+	+	–	–	ND
D-Glucose	+	+	+	+	+	–	ND
D-Lactose	–	+	+	+	–	–	ND
D-Maltose	+	–	+	+	+	–	ND
D-Melibiose	+	+	+	+	–	–	ND
L-Raffinose	+	–	+	+	–	–	ND
L-Rhamnose	+	+	+	+	–	–	ND
D-Sucrose	–	+	+	+	+	+	ND
DL-Xylose	+	+	+	+	+	–	ND
N-Acetylglucosamine	+	+	–	+	+	–	ND
Utilization of:							
Mannitol	+	+	–	–	–	–	–
Sorbitol, inositol, malonate	+	–	–	–	–	–	–
Fumarate, malate	V	–	–	–	–	–	ND
Susceptibility to:							
Ampicillin	–	–	–	–	–	+	ND
Benzylpenicillin	–	–	–	–	–	+	ND
Carbenicillin	+	+	–	V	+	+	ND
Lincomycin	+	+	+	V	+	+	ND
Oleandomycin	+	+	–	+	+	+	ND
Streptomycin	–	–	–	–	–	+	ND
Tetracycline	+	+	–	V	+	+	ND
DNA G+C content (mol%)	35–37	41.3	37–40.5	39–42	37	42	38

different from the other two marine strains studied by the ability to reduce nitrates to nitrites, growth at 39 °C, absence of growth above 6 % NaCl and hydrolysis of starch. Strain KMM 3957^T was distinguished from other strains studied by hydrolysis of gelatin, requirement of NaCl for growth, absence of acid production from *N*-acetylglucosamine and resistance to carbenicillin and oleandomycin. Strain KMM 3958^T was distinguished from the above-mentioned strains by the hydrolysis of casein, DNA and Tween 80 and the inability to produce acid from galactose and maltose and to hydrolyse agar. The genomic data in combination with differentiating phenotypic data (Table 2) confirm that KMM 3956^T, KMM 3957^T and KMM 3958^T represent three separate species.

We conclude that the data presented indicate that marine bacteria KMM 3956^T, KMM 3957^T and KMM 3958^T represent three novel *Algoriphagus* species, for which we propose the names *Algoriphagus winogradskyi* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus aquimarinus* sp. nov., respectively. Based on phylogenetic and chemotaxonomic data, we further propose that *Hongiella halophila* be reclassified as *Algoriphagus halophilus* comb. nov. The new species descriptions and species reclassification justify emendation of the descriptions of the genera *Algoriphagus* and *Hongiella*.

Emended description of the genus *Hongiella* Yi and Chun 2004

Hongiella (Hong.i.el'la. N.L. dim. fem. n. *Hongiella* named after Soon-Woo Hong, a Korean microbiologist who devoted his life to the study of soil micro-organisms).

Rod-shaped cells, non-motile. Gram-negative. Does not form endospores. May require Na⁺ ions for growth. Strictly aerobic. Produces non-diffusible pink pigments. No flexirubins are formed. Chemo-organotroph. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Can reduce nitrates to nitrites. Main cellular fatty acids are 15:0iso, 16:0iso, 17:1ω6c, 17:0iso 3-OH and 17:1isoω9c. The major isoprenoid quinone is MK-7. The DNA G + C content is 38–43 mol%. As determined by 16S rRNA gene sequence analysis, the genus *Hongiella* is a member of the phylum *Bacteroidetes*.

The type species is *Hongiella mannitolivorans*.

Emended description of the genus *Algoriphagus* Bowman et al. 2003

Algoriphagus (Al.go.ri.pha'gus. L. masc. n. *algor* cold; Gr. masc. n. *phagos* glutton; N.L. masc. n. *Algoriphagus* the cold eater).

Rod-shaped cells, non-motile. Gram-negative. Does not form endospores. May require Na⁺ ions for growth. Strictly aerobic. Produces non-diffusible pink pigments. No flexirubins are formed. Chemo-organotroph. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Can

hydrolyse agar, gelatin, starch and DNA. Can reduce nitrates to nitrites. The main cellular fatty acids are 15:0iso, 17:0iso 3-OH and summed feature 3. The major isoprenoid quinone is MK-7. The DNA G + C content is 35–42 mol%. As determined by 16S rRNA gene sequence analysis, the genus *Algoriphagus* is a member of the phylum *Bacteroidetes*.

The type species is *Algoriphagus ratkovskyi*. Inhabits sea ice and sea water, algae, marine sediments.

Description of *Algoriphagus aquimarinus* sp. nov.

Algoriphagus aquimarinus (a.qui.ma.ri'nus. L. fem. n. *aqua* water, L. adj. *marinus*-, -a, -um marine, of the sea; N.L. masc. adj. *aquimarinus* of sea water).

Main characteristics are the same as those given for the genus. In addition, cells range from 0.5 to 0.7 µm in width and 2 to 10 µm in length. On marine agar, colonies are 2–3 mm in diameter, circular, shiny with entire edges and are pale-pink pigmented. Growth occurs at 4–34 °C (optimum 23–25 °C). Growth occurs at 0–10 % NaCl. Decomposes casein, gelatin, aesculin, alginate, DNA and Tweens 20, 40 and 80. Does not hydrolyse agar, starch, cellulose (CM-cellulose and filter paper) or chitin. Forms acid from D-cellobiose, L-fucose, D-glucose, D-lactose, D-melibiose, L-rhamnose, D-sucrose, DL-xylose and *N*-acetylglucosamine, but not from L-arabinose, D-galactose, D-maltose, L-raffinose, L-sorbose, adonitol, glycerol or mannitol. Utilizes L-arabinose, D-glucose, D-lactose, D-mannose, D-sucrose and mannitol, but not inositol, sorbitol, malonate or citrate. Produces β-galactosidase. Nitrate is not reduced. H₂S, indole and acetoin (Voges–Proskauer reaction) production are negative. Susceptible to carbenicillin, lincomycin, oleandomycin and tetracycline; resistant to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. The predominant fatty acids are 15:0iso, 16:1ω5c, 17:0iso 3-OH, 17:1isoω9c and summed feature 3. The G + C content of the DNA is 41 mol%.

The type strain is KMM 3958^T (=LMG 21971^T=CCUG 47101^T). Isolated from a sea-water sample.

Description of *Algoriphagus chordae* sp. nov.

Algoriphagus chordae (chor'dae. N.L. gen. n. *chordae* of *Chorda*, the generic name of the brown alga *Chorda filum*, from which the type strain was isolated).

Main characteristics are the same as those given for the genus. In addition, cells range from 0.5 to 0.7 µm in width and 2 to 10 µm in length. On marine agar, colonies are 2–3 mm in diameter, circular, shiny with entire edges, bright-pink pigmented, compressed into the agar. Growth occurs at 4–32 °C (optimum 23–25 °C). Growth occurs at 1–10 % NaCl. Decomposes agar, alginate, Tween 20 and Tween 40. Does not hydrolyse casein, gelatin, starch, DNA,

Tween 80, cellulose (CM-cellulose and filter paper) or chitin. Forms acid from D-cellobiose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, DL-xylose and D-sucrose, but not from L-arabinose, L-sorbose, *N*-acetylglucosamine, adonitol, glycerol or mannitol. Utilizes arabinose, glucose, lactose, mannose, sucrose, but not mannitol, inositol, sorbitol, malonate or citrate. Produces β -galactosidase. Nitrate is not reduced. H₂S, indole and acetoin (Voges–Proskauer reaction) production are negative. Susceptible to lincomycin; resistant to ampicillin, benzylpenicillin, carbenicillin, gentamicin, kanamycin, neomycin, polymyxin B, oleandomycin, tetracycline and streptomycin. The predominant fatty acids are 15:0iso, 16:0iso, 17:0iso 3-OH and summed feature 3. The G + C content of the DNA is 37–40 mol%.

The type strain is KMM 3957^T (=LMG 21970^T=CCUG 47095^T). Isolated from the brown alga *Chorda filum*.

Description of *Algoriphagus winogradskyi* sp. nov.

Algoriphagus winogradskyi (wi.no.grad'sky.i. N.L. gen. n. *winogradskyi* of Winogradsky, to honour Sergey N. Winogradsky, for his contributions to the study of *Cytophaga*-like bacteria).

Main characteristics are the same as those given for the genus. In addition, cells range from 0.5 to 0.7 μ m in width and 2 to 10 μ m in length. On marine agar, colonies are 2–4 mm in diameter, circular, shiny with entire edges, bright-pink pigmented, weakly compressed into the agar. Growth occurs at 4–39 °C (optimum 25–28 °C). Growth occurs at 0–6 % NaCl. Decomposes agar, gelatin, aesculin, starch, alginate, Tween 20 and Tween 40. Does not hydrolyse casein, DNA, Tween 80, cellulose (CM-cellulose and filter paper) or chitin. Forms acid from D-cellobiose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, D-sucrose, DL-xylose and *N*-acetylglucosamine, but not from L-arabinose, L-fucose, L-sorbose, adonitol, glycerol or mannitol. Utilizes L-arabinose, D-glucose, D-lactose, D-mannose, D-sucrose, but not mannitol, inositol, sorbitol, malonate or citrate. Produces β -galactosidase. Nitrate is reduced. H₂S, indole and acetoin (Voges–Proskauer reaction) production are negative. Susceptible to carbenicillin, lincomycin, oleandomycin and tetracycline; resistant to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. The predominant fatty acids are 15:0iso, 17:0iso 3-OH and summed feature 3. The G + C content of the DNA is 39–42 mol%.

The type strain is KMM 3956^T (=LMG 21969^T=CCUG 47094^T). Isolated from the green alga *Acrosiphonia sonderi*.

Description of *Algoriphagus halophilus* (Yi and Chun 2004) comb. nov.

Algoriphagus halophilus (ha.lo.phi'lus. Gr. n. *halos* salt; Gr. adj. *philos* loving; N.L. masc. adj. *halophilus* salt-loving).

Basonym: *Hongiella halophila* Yi and Chun 2004.

The description is as given by Yi & Chun (2004) with the addition that the organism grows at 10–41 °C and at 0–8 % NaCl. Colonies are bright pink. Hydrolyses starch. Forms acid from D-glucose, D-maltose, DL-xylose and *N*-acetylglucosamine, but not from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-lactose, D-melibiose, L-raffinose, L-rhamnose, L-sorbose, adonitol, glycerol or mannitol. Utilizes L-arabinose, but not malonate. Susceptible to carbenicillin, lincomycin, oleandomycin and tetracycline; resistant to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, streptomycin and polymyxin B. The predominant fatty acids are 15:0iso, 16:0iso, 17:1iso ω 9c, 17:0iso 3-OH and summed feature 3.

The type strain is JC 2051^T (=KCTC 12051^T=DSM 15292^T).

Note added in proof

Since this study was completed, Yoon *et al.* (2004) have described a novel species of the genus *Hongiella*, *Hongiella marincola*, that is characterized by the inability to grow without Na⁺ ions or sea water and has a DNA G + C content of 43 mol%. These data were added in proof to the emended description of the genus *Hongiella*.

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References

- Akagawa-Matsushita, M., Itoh, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992).** Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Bowman, J. P. (2000).** Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.
- Bowman, J. P., Nichols, C. M. & Gibson, J. A. E. (2003).** *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. *Int J Syst Evol Microbiol* **53**, 1343–1355.
- Brettar, I., Christen, R. & Höfle, M. G. (2004).** *Belliella baltica* gen. nov., sp. nov., a novel marine bacterium of the *Cytophaga*–*Flavobacterium*–*Bacteroides* group isolated from surface water of the central Baltic Sea. *Int J Syst Evol Microbiol* **54**, 65–70.

- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Lemos, M. L., Toranzo, A. E. & Barja, J. L. (1985). Modified medium for oxidation-fermentation test in the identification of marine bacteria. *Appl Environ Microbiol* **40**, 1541–1543.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.
- Niemann, S., Puehler, A., Tichy, H.-V., Simon, R. & Selbitschka, W. (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. *J Appl Microbiol* **82**, 477–484.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Raj, H. D. & Maloy, S. R. (1990). Proposal of *Cyclobacterium marinus* gen. nov., comb. nov. for a marine bacterium previously assigned to the genus *Flectobacillus*. *Int J Syst Bacteriol* **40**, 337–347.
- Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.
- Yi, H. & Chun, J. (2004). *Hongiella mannitolivorans* gen. nov., sp. nov., *Hongiella halophila* sp. nov. and *Hongiella ornithinivorans* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 157–162.
- Yoon, J.-H., Yeo, S.-H. & Oh, T.-K. (2004). *Hongiella marincola* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1845–1848.