Wandonia haliotis gen. nov., sp. nov., a marine bacterium of the family *Cryomorphaceae*, phylum *Bacteroidetes*

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A novel, strictly aerobic, Gram-stain-negative, yellow-orange-pigmented, rod-shaped bacterium was isolated from abalone (*Haliotis discus*) under aquaculture in seawater off the Wando coast, Southern Korea, and subjected to a polyphasic taxonomic study. Cells of strain Haldis-1^T were catalase- and oxidase-positive rods with flexirubin pigments. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Haldis-1^T formed a distinct lineage within the family *Cryomorphaceae* and could be distinguished from the related genera *Lishizhenia* and *Fluviicola*. Strain Haldis-1^T shared 16S rRNA gene sequence similarities of 92.5 and 92.4 % with *Lishizhenia caseinilytica* UST040201-001^T and *Fluviicola taffensis* RW262^T, respectively. The DNA G + C content was 38.1 mol% and the major respiratory quinone was MK-7. The predominant cellular fatty acids were iso-C_{15:0} (38.6 %), C_{15:0} 2-OH (20.3 %) and C_{15:0} (10.7 %). Growth was observed at 25–42 °C (optimum 30–37 °C) and at pH 6.5–9.5 (optimum pH 6.5–8.0). On the basis of polyphasic analysis of phenotypic, genotypic and phylogenetic data, strain Haldis-1^T represents a novel genus and species within the family *Cryomorphaceae* in the phylum *Bacteroidetes*, for which the name *Wandonia haliotis* gen. nov., sp. nov. is proposed. The type strain is Haldis-1^T (=KCTC 22610^T =NBRC 105642^T).

The family *Cryomorphaceae*, within the phylum *Bacteroidetes*, includes five genera of marine origin: *Brumimicrobium*, *Cryomorpha*, *Crocinitomix* (Bowman *et al.*, 2003), *Owenweeksia* (Lau *et al.*, 2005) and *Lishizhenia* (Lau *et al.*, 2006), and the freshwater genus *Fluviicola* (O'Sullivan *et al.*, 2005). Members of the family *Cryomorphaceae* cannot utilize carbohydrates and require complex organic compounds for growth (Bowman *et al.*, 2003; Lau *et al.*, 2005; O'Sullivan *et al.*, 2005). Molecular phylogenetic studies have found that phylotypes related to the family *Cryomorphaceae* are associated with phytoplankton blooms (Pinhassi *et al.*, 2004; Grossart *et al.*, 2005). In the process of investigating bacterial communities in abalone (*Haliotis discus*) under aquaculture for food, a novel Gram-stain-negative bacterium producing yellow-orange pigments was isolated and investigated using a polyphasic approach.

Strain Haldis-1^T was isolated from abalone flesh samples collected off the southern coast of Wando (34° 18' N 126° 45' E) in Korea by using a standard serial dilution plating method and incubation on marine agar 2216 (MA; Difco) at 25 °C for 5 days. Subcultivation was routinely performed on LB agar at 30 °C for 3 days under aerobic conditions and the strain was stored at –80 °C in marine broth (MB; Difco) supplemented with 15 % (v/v) glycerol for preservation.

Sequencing of the 16S rRNA gene of strain Haldis-1^T was carried out as described by Lane (1991). The almost full-length (1480 nt) 16S rRNA gene sequence was compared with 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 organisms by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony algo-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Haldis-1^T is FJ424814.

rithms available in the PHYLIP software package, version 3.6 (Felsenstein, 2002). The resulting tree topologies were evaluated by using bootstrap analysis based on 1000 re-sampled datasets with the PHYLIP package. Sequence similarity values between the novel isolate and related strains were evaluated using the Nucleotide Similarity Search program (<http://www.ebi.ac.uk/fasta33/nucleotide.html>; Pearson & Lipman, 1988). Neighbour-joining analysis indicated that strain Haldis-1^T formed a distinct phylogenetic line within the family *Cryomorphaceae*, related to the genera *Lishizhenia*, *Fluviicola*, *Brumimicrobium*, *Crocinitomix* and *Owenweeksia* with a low bootstrap value, meaning that the phylogenetic topology is not stable (Fig. 1). Comparative analysis of 16S rRNA gene sequences showed that the isolate was most closely related to *Lishizhenia caseinilytica* UST040201-001^T and *Fluviicola taffensis* RW262^T with 92.5 and 92.4 % sequence similarity, respectively. These data suggested that strain Haldis-1^T represents a novel genus within the family *Cryomorphaceae*.

The following tests were performed on strain Haldis-1^T. Growth temperature was examined on LB agar at 5–45 °C in 5 °C increments. The pH range for growth was determined in LB broth adjusted to pH 5.0–10.0 in 0.5 pH unit increments. The pH was adjusted prior to

sterilization by the addition of HCl or NaOH and measured again after sterilization. NaCl requirement and tolerance were determined in the presence of 0–10 % (w/v) NaCl (at 1 % intervals) in LB broth. Gram staining was performed using the bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology and flagellar and gliding motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described previously (Bernardet *et al.*, 2002; Jeon *et al.*, 2004). Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. The production of flexirubin-type pigments was investigated using the KOH test according to the method of Fautz & Reichenbach (1980). Nitrate reduction was assessed according to the method of Lányi (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Anaerobic growth was assessed on LB agar under anaerobic (with 4–10 % CO₂) conditions using the GasPak Plus system (BBL) at 30 °C for 15 days. Hydrolysis of aesculin, Tween 80, Tween 20, casein, urea, tyrosine, starch and CM-cellulose was investigated on LB agar after 7 days of incubation at 30 °C,

Fig. 1. Neighbour-joining phylogenetic tree reconstructed based on 16S rRNA gene sequences showing the relationships of strain Haldis-1^T and related taxa within the phylum *Bacteroidetes*. Bootstrap percentages (based on 1000 replicates) greater than 50 % are shown at branch points. *Escherichia coli* ATCC 11775^T (accession no. X80725) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

according to the methods described by Lányi (1987) and Smibert & Krieg (1994). Additional enzymic activities were determined using the API ZYM kit, as recommended by the manufacturer (bioMérieux), with incubation for 2 days at 30 °C. Antibiotic susceptibility tests were performed in duplicate as described by Reva *et al.* (1995) 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 U), chloramphenicol (100 µg), gentamicin (30 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), carbenicillin (100 µg) and novobiocin (50 µg). The phenotypic characteristics of strain Haldis-1^T are listed in Table 1 and in the genus and species descriptions.

The total cellular fatty acid composition of strain Haldis-1^T was analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) using cells grown on tryptic soy agar at 30 °C for 3 days (Table 2). Analysis of isoprenoid quinones was carried out using the methods described by Komagata & Suzuki (1987). The DNA G+C content of strain Haldis-1^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). The major respiratory lipoquinone was menaquinone-7 (MK-7),

which was distinct from the menaquinone-6 (MK-6) found in *Owenweeksia hongkongensis* UST20020801^T of the family *Cryomorphaceae*.

On the basis of phenotypic, genotypic and phylogenetic data, strain Haldis-1^T is considered to represent a novel genus and species within the family *Cryomorphaceae*, for which the name *Wandonia haliotis* gen. nov., sp. nov. is proposed.

Description of *Wandonia* gen. nov.

Wandonia (Wan.do'ni.a. N.L. fem. n. *Wandonia* named after Wando, an island located on the Southern Sea in Korea, from where the type strain of the type species was isolated).

Cells are strictly aerobic, Gram-stain-negative, rod-shaped. Oxidase- and catalase-positive. Cell mass is pigmented yellow-orange and flexirubins are synthesized. Cells do not utilize carbohydrate. The cellular fatty acid profiles contain a high proportion of branched saturated fatty acids (mainly iso-C_{15:0}), hydroxy fatty acids (mainly C_{15:0} 2-OH), and saturated fatty acids (C_{15:0}). As determined by 16S rRNA gene sequence analysis, the genus *Wandonia* is a member of the family *Cryomorphaceae* in the phylum *Bacteroidetes*. The type species is *Wandonia haliotis*.

Table 1. Phenotypic characteristics that differentiate strain Haldis-1^T from related type species of genera of the family *Cryomorphaceae*

Strains: 1, Haldis-1^T (data from this study); 2, *Fluviicola taffensis* RW262^T (data from O'Sullivan *et al.*, 2005); 3, *Lishizhenia caseinilytica* UST040201-001^T (data from Lau *et al.*, 2006); 4, *Brumimicrobium glaciale* IC156^T (data from Bowman *et al.*, 2003); 5, *Crocinitomix catalasitica* NCIMB 1418^T (data from Bowman *et al.*, 2003). +, Positive; –, negative; v, variable; w, weakly positive; ND, data not available; A, aerobic; F, facultatively anaerobic.

Characteristic	1	2	3	4	5
Habitat	Marine	Fresh water	Marine	Marine	Marine
Cell size (µm)	1–2	1.5–5.7	0.5–3.8	1–3	3–100
Colour of cell mass	Yellow-orange	Yellow-orange	Orange	Orange	Yellow
Gliding motility	+	+	–	–	–
Growth on/at:					
25 °C	+	+	+	v/w	+
42 °C	+	–	–	–	–
1 % NaCl	+	–	+	+	+
6 % NaCl	+	–	+	–	–
Metabolism	A	A	A	F	A
Oxidase	+	–	+	–	–
Flexirubin reaction	+	+	–	–	–
Glucose utilization	–	–	–	+	–
Degradation of:					
Gelatin	+	w	+	–	–
Casein	+	ND	+	–	–
DNase	–	+	–	–	–
Phosphatase	+	ND	+	+	–
Nitrate reductase	–	–	–	+	–
DNA G+C content (mol%)	38.1	37.2	35.8	38–40	35

Table 2. Cellular fatty acids that differentiate strain Haldis-1^T from the type species of genera of the family *Cryomorphaceae*

Strains: 1, Haldis-1^T (data from this study); 2, *Fluviicola taffensis* RW262^T (data from O'Sullivan *et al.*, 2005); 3, *Lishizhenia caseinilytica* UST040201-001^T (data from Lau *et al.*, 2006); 4, *Brumimicrobium glaciale* IC156^T (data from Bowman *et al.*, 2003); 5, *Crocinitomix catalastica* NCIMB 1418^T (data from Bowman *et al.*, 2003). Fatty acids amounting to less than 0.5% in all species are not shown. tr, Trace amount (<0.5%); –, not detected.

Fatty acid	1	2	3	4	5
Saturated					
C _{13:0}	1.0	tr	tr	0.5	–
C _{14:0}	4.3	3.2	1.6	1.5	tr
C _{15:0}	10.7	7.5	–	2.9	29.3
C _{16:0}	1.3	3.0	0.5	1.9	1.8
Saturated branched-chain					
iso-C _{15:0}	38.6	44.2	44.0	36.4	36.3
iso-C _{15:1}	–	–	34.8	–	–
iso-C _{15:1} G	3.7	–	–	–	–
Unsaturated branched-chain					
iso-C _{15:1} ω10c	–	11.8	–	–	10.9
anteiso-C _{15:1} ω10c	–	–	–	45.0	–
Monounsaturated					
C _{15:1} ω11c	–	1.2	–	–	8.3
C _{16:1} ω12c	–	4.9	–	–	4.4
Hydroxy					
C _{14:0} 2-OH	1.8	–	–	–	–
C _{15:0} 2-OH	20.3	–	–	–	–
C _{16:0} 2-OH	1.1	–	–	–	–
iso-C _{15:0} 2-OH	–	3.5	–	–	–
C _{15:0} 3-OH	1.7	–	–	–	–
iso-C _{15:0} 3-OH	–	0.5	2.5	–	1.0
C _{16:0} 3-OH	2.1	–	0.5	–	–
iso-C _{17:0} 3-OH	5.0	12.3	9.3	–	3.3
Summed feature 3*	8.4	–	2.0	–	–

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1} ω7c and/or iso-C_{15:0} 2-OH.

Description of *Wandonia haliotis* sp. nov.

Wandonia haliotis (ha.li.o'tis. N.L. n. *Haliotis* scientific name of a genus; N.L. gen. n. *haliotis* of *Haliotis*, referring to the isolation of the type strain from *Haliotis discus*).

Exhibits the following characteristics in addition to those given in the genus description. Cells are 0.2–0.3 µm wide and 2–5 µm long. On LB medium, colonies are 1.0–3.0 mm in diameter, circular, flat and transparent. Catalase- and oxidase-positive. Growth occurs at 25–42 °C, with an optimum at 30–37 °C. Negative for nitrate and nitrite reduction. Indole is not produced. Negative for β-galactosidase and urease activity. Can hydrolyse casein and gelatin, but not DNA, agar, starch, cellulose or aesculin. Acid is not produced from glucose. Does not utilize glucose, arabinose, mannose, mannitol, *N*-acetyl-

glucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenyl acetate. In the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Resistant to gentamicin (10 µg), kanamycin (50 µg), polymyxin B (300 U), neomycin (30 µg) and novobiocin (5 µg), but sensitive to ampicillin (10 µg), carbenicillin (100 µg), erythromycin (15 µg), lincomycin (15 µg), nalidixic acid (5 µg), oleandomycin (15 µg), penicillin G (10 U) and tetracycline (30 µg). The major (>5% of total) cellular fatty acids are iso-C_{15:0}, C_{15:0} 2-OH, C_{15:0} and summed feature 3 (comprising C_{16:1} ω7c and/or iso-C_{15:0} 2-OH). The DNA G+C content of the type strain is 38.1 mol%.

The type strain is Haldis-1^T (=KCTC 22610^T =NBRC 105642^T), which was isolated from abalone (*Haliotis discus*) under aquaculture in seawater off the Wando coast, Southern Korea.

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