Haloferula rosea gen. nov., sp. nov., *Haloferula harenae* sp. nov., *Haloferula phyci* sp. nov., *Haloferula helveola* sp. nov. and *Haloferula sargassicola* sp. nov., five marine representatives of the family *Verrucomicrobiaceae* within the phylum '*Verrucomicrobia*'

Jaewoo Yoon,¹ Yoshihide Matsuo,² Atsuko Katsuta,² Jae-Hyuk Jang,^{2†} Satoru Matsuda,² Kyoko Adachi,² Hiroaki Kasai² and Akira Yokota¹

Correspondence

Jaewoo Yoon

aa57058@mail.ecc.u-tokyo.ac.jp

¹Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-0032, Japan

²Marine Biotechnology Institute Co. Ltd, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan

Six Gram-negative, non-motile, rod-shaped, chemoheterotrophic bacteria designated strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 were isolated from various marine environments and subjected to a polyphasic taxonomic study. Preliminary analysis based on 16S rRNA gene sequences revealed that the novel isolates could be affiliated with the family *Verrucomicrobiaceae* of the phylum '*Verrucomicrobia*'. The strains shared approximately 94–100% sequence similarity with each other and showed less than 94% similarity with members of the family *Verrucomicrobiaceae* with validly published names. The DNA–DNA relatedness between strains YM23-227^T and 06SJR1-1^T was less than 70%, a value that is accepted as a phylogenetic definition of a species. The cell wall peptidoglycan of the strains contained muramic acid and meso-diaminopimelic acid. The novel isolates produced carotenoid pigments and squalene. The DNA G + C contents of the six strains were 63–65 mol%. The major menaquinone was MK-9 and iso-C_{14:0} was the major fatty acid. Based on the evidence from the polyphasic taxonomic study, it was concluded that the six strains should be classified as representing a new genus and five novel species of the family *Verrucomicrobiaceae* within the phylum '*Verrucomicrobia*', for which the names *Haloferula rosea* gen. nov., sp. nov. (type strain 06SJR1-1^T=MBIC08340^T=KCTC 22201^T), *Haloferula harenae* sp. nov. (type strain YM23-227^T=MBIC08299^T=KCTC 22198^T), *Haloferula phyci* sp. nov. (type strain AK18-024^T=MBIC08341^T=KCTC 22200^T), *Haloferula helveola* sp. nov. (type strain 05IJR53-1^T=MBIC08342^T=KCTC 22199^T) and *Haloferula sargassicola* sp. nov. (type strain MN1-1037^T=MBIC08343^T=KCTC 22202^T) are proposed.

Many culture-independent studies based on 16S rRNA gene sequences have revealed that members of the phylum '*Verrucomicrobia*' (Hedlund *et al.*, 1997a; Hugenholtz *et al.*, 1998) thrive in a wide range of terrestrial, aquatic and marine habitats (Joseph *et al.*, 2003; Rappé & Giovannoni, 2003; Kanokratana *et al.*, 2004; Haukka *et al.*, 2005, 2006; Dedysch *et al.*, 2006). This phylogenetic group, which is one of the

primary lineages of domain *Bacteria*, has been classified informally into six monophyletic subdivisions (Hugenholtz *et al.*, 1998; Vandekerckhove *et al.*, 2000). Among them, subdivision 1 has been recognized as the family *Verrucomicrobiaceae* in the second edition of *Bergey's Manual of Systematic Bacteriology* (Garrity & Holt, 2001). Recently, for the formal classification of the phylum '*Verrucomicrobia*', emended descriptions of subdivision 1 were presented as the class *Verrucomicrobiae*, which is composed of one order, *Verrucomicrobiales*, and one family, *Verrucomicrobiaceae*, with six novel marine representatives (Yoon *et al.*, 2008b).

The strains investigated and source samples are listed in Table 1. Strain YM23-227^T was isolated from sediment

†Present address: Center for Marine Natural Products and Drug Discovery, Seoul National University, Seoul 151-747, Republic of Korea.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 are AB372852, AB372853, AB372854, AB372855, AB372856 and AB372857, respectively.

Table 1. Strains investigated in this study

Strain	Source	Sampling site (GPS location)	Medium for isolation	Date
YM23-227 ^T	Sediment	Milky Way marine lake, Republic of Palau (7° 15' 49.2'' N 134° 24' 55.7'' E)	R*	December 2005
06SJR1-1 ^T	Sponge, unidentified	Cape Nagate, Sado Island, Niigata, Japan (37° 58' 37.3'' N 138° 13' 45.3'' E)	1/10-strength MA containing 1 % rose Bengal	August 2006
AK18-024 ^T	Green algae, unidentified	Hoshinosuna beach, Iriomote Island, Okinawa, Japan (24° 26' 8.1'' N 123° 46' 39.9'' E)	1/100-strength MA with dibenzothiophen vapour†	October 2005
05IJR53-1 ^T	Seawater	Estuary of Urauchi River, Iriomote Island, Okinawa, Japan (24° 24' 15.4'' N 123° 46' 40.1'' E)	1/10-strength MA containing 0.5 % rose Bengal	October 2005
MN1-1037 ^T	Brown alga, <i>Sargassum thunbergii</i> (Mertens ex Roth) Kuntze	Abajima, Takehara, Hiroshima, Japan (34° 19' 13'' N 132° 56' 26'' E)	M1‡ plus cycloheximide (50 µg ml ⁻¹), nalidixic acid (40 µg ml ⁻¹) and rifampicin (5 µg ml ⁻¹)	September 2006
MN1-1047	Brown alga, <i>Sargassum thunbergii</i> (Mertens ex Roth) Kuntze	Abajima, Takehara, Hiroshima, Japan (34° 19' 13'' N 132° 56' 26'' E)	M1 plus cycloheximide (50 µg ml ⁻¹), nalidixic acid (40 µg ml ⁻¹), fradiomycin (50 µg ml ⁻¹) and aztreonam (50 µg ml ⁻¹)	September 2006

*The composition of medium R is given in Supplementary Table S1 with Yoon *et al.* (2008b).

†Dibenzothiophen was fed as vapour generated from 35 mg dibenzothiophen powder on the lid of a Petri dish.

‡Composition of medium M1 is described by Mincer *et al.* (2002).

collected from the Milky Way marine lake (GPS location: 7° 15' 49.2'' N 134° 24' 55.7'' E) in the Republic of Palau, in December 2005. The sediment sample (1 g) was homogenized with a glass rod in 5 ml sterile seawater. The homogenate (50 µl) was applied to medium R agar (Yoon *et al.*, 2008b) for isolation. Strain YM23-227^T appeared as a tiny pink colony after incubation for 30 days at room temperature (25 °C). Strain 06SJR1-1^T was isolated from an unidentified sponge collected at Cape Nagate in Sado Island (GPS location: 37° 58' 37.3'' N 138° 13' 45.3'' E) in Niigata, Japan in August 2006. The sponge sample (1 cm³) was homogenized with a glass rod in 5 ml sterile seawater and the homogenate (50 µl) was applied to 1/10-strength marine agar 2216 (MA; Difco) containing 1 % rose Bengal for isolation. The agar medium was irradiated by sunlight for at least 24 h to activate the rose Bengal. Strain 06SJR1-1^T appeared as a tiny colony after incubation for 14 days at room temperature. Strain AK18-024^T was isolated from unidentified green algae collected at Hoshinosuna beach, Iriomote Island (GPS location: 24° 26' 8.1'' N 123° 46' 39.9'' E) in Okinawa, Japan in October 2005. A piece of the algae (1 cm³) was homogenized with a glass rod in 5 ml sterile seawater. The homogenate (50 µl) was applied to 1/100-strength MA with dibenzothiophen vapour for isolation. The dibenzothiophen vapour was generated from 35 mg dibenzothiophen powder on the lid of a Petri dish. Strain AK18-024^T appeared as a tiny orange colony after incubation for 5 months at room temperature. Strain 05IJR53-1^T was isolated from seawater collected at the estuary of the Urauchi River (GPS location: 24° 24' 15.4'' N 123° 46' 40.1'' E) in Okinawa, Japan in October 2005. The seawater sample (50 µl) was applied to 1/10-strength MA containing 0.5 % rose Bengal for isolation. The agar medium was irradiated by sunlight for at least 1 h to activate the rose Bengal. Strain 05IJR53-1^T appeared as a tiny colony after incubation for 14 days at room temperature. Strains MN1-1037^T and MN1-1047 were isolated from brown algae, *Sargassum thunbergii* (Mertens ex Roth) Kuntze, collected at Abajima (GPS location: 34° 19' 13'' N 132° 56' 26'' E) in Takehara, Hiroshima, Japan in September 2006. A piece of algae (1 cm³) was homogenized with a glass rod in 5 ml sterile seawater. The homogenate (50 µl) was applied to M1 agar medium for isolation. Strain MN1-1037^T appeared as a tiny pale-orange colony after incubation at room temperature for 45 days on M1 medium (Mincer *et al.*, 2002), containing cycloheximide (50 µg ml⁻¹), nalidixic acid (40 µg ml⁻¹), rifampicin (5 µg ml⁻¹) and 1.5 % agar. Strain MN1-1047 appeared as a tiny pale-orange colony after incubation at room temperature for 60 days on M1 medium containing cycloheximide (50 µg ml⁻¹), nalidixic acid (40 µg ml⁻¹), fradiomycin (50 µg ml⁻¹), aztreonam (50 µg ml⁻¹) and 1.5 % agar.

In this study, we attempted to elucidate the phylogenetic position of strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 using a polyphasic taxonomic approach, including 16S rRNA gene sequence

analysis. In parallel, we performed physiological, biochemical and chemotaxonomic analyses to characterize the six novel isolates. Based on these data, it is proposed that the isolates represent a new genus and five novel species of the family *Verrucomicrobiaceae* within the phylum 'Verrucomicrobia'.

The temperature (4–45 °C) and pH (5.5–9.5) ranges for growth were determined by incubating the isolates on MA and 1/5-strength MA. The NaCl concentration for growth was determined on 1/2-strength R2A agar (Difco) containing 0–10 % (w/v) NaCl and a salt tolerance test medium containing 1 % tryptone, 0.3 % yeast extract, 0.9 % $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.9 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 % $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.06 % KCl and 1.5 % agar with 0–10 % (w/v) NaCl. Gram-staining was performed as described by Murray *et al.* (1994). Cell morphology was observed using light microscopy (BX60; Olympus). Growth under anaerobic conditions was determined after incubation for 2 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co.) on 1/5-strength MA and MA. Catalase activity was determined by bubble formation in a 3 % H_2O_2 solution. Oxidase activity was determined using cytochrome oxidase test paper (Nissui Pharmaceutical Co.). API 20NE and API ZYM strips (bioMérieux) were used to determine the physiological and biochemical characteristics. All suspension media for the API test strips were supplemented with 0.85 % (w/v) NaCl solution (final concentration). API 20NE and API ZYM test strips were read after 48 h incubation at 30 °C and 4 h incubation at 37 °C, respectively. The substrate oxidation ranges of the strains were determined using Biolog MicroPlates. The strains were grown on 1/5-strength MA and MA medium at 30 °C for 96 h and suspended in sterile saline medium (0.85 % NaCl, w/v) within the density range specified by the manufacturer using a Biolog photometer (model 21907). Immediately after the cells had been suspended in saline solution, the suspensions were transferred to sterile multi-channel pipetter reservoirs (Biolog) and the Biolog GN2 MicroPlates were inoculated with 150 µl cell suspension per well by means of an eight-channel repeating pipetter (Biolog). The inoculated plates were incubated at 30 °C for 24 h, and the results were read with a MicroPlate Reader using Microlog 3.59 computer software for automated reading. Determination of the respiratory quinone system and cellular fatty acid composition were carried out as described previously (Katsuta *et al.*, 2005). DNA was prepared according to the method of Marmur (1961) from cells grown on 1/5-strength MA and MA medium and the DNA base composition was determined by using the HPLC method of Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989). The hybridization temperature was set at 53 °C. Hybridization was performed using five replications for each. Of the values obtained, the highest and lowest for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values. Cell walls

were prepared by using the methods described by Schleifer & Kandler (1972) and amino acids in an acid hydrolysate of the cell walls were identified using TLC (Harper & Davis, 1979) and HPLC, as their phenylthiocarbamoyl derivatives, with a model LC-10AD HPLC apparatus (Shimadzu) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries) (Yokota *et al.*, 1993). An approximately 1500 bp fragment of the 16S rRNA gene was amplified from the extracted DNA by using bacterial universal primers specific to the 16S rRNA gene: 27F and 1492R (*Escherichia coli* numbering system; Weisburg *et al.*, 1991). To ascertain the phylogenetic position of the novel isolates, the 16S rRNA gene sequences of strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 were compared with sequences obtained from GenBank (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>). Multiple alignments of the sequences were performed using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were not taken into consideration when the 1121 bases of 16S rRNA gene nucleotides were compared. Aligned sequences were analysed by using MEGA3.1 software (Kumar *et al.*, 2004). The evolutionary distances [distance options according to the Kimura two-parameter model (Kimura, 1983)] and clustering with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods were determined by using bootstrap values based on 1000 replications (Felsenstein, 1985). The similarity values were calculated using the same software.

The phylogenetic tree based on neighbour-joining- and maximum-parsimony-generated comparisons of the 16S rRNA gene sequences revealed that the strains studied form a different monophyletic clade within the family *Verrucomicrobiaceae* with bootstrap confidence values of 98 % in the neighbour-joining method (Fig. 1) and more than 70 % in the maximum-parsimony method (data not shown). Strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 formed a single cluster with sequence similarities between the strains ranging from 94.7 to 100 % (Fig. 1). Strains YM23-227^T and 06SJR1-1^T were 98.2 % similar to each other whereas the other strains were slightly more distant. In addition, the 16S rRNA gene sequence similarity between strains MN1-1037^T and MN1-1047 was 100 %. All other so far cultivated species of the family *Verrucomicrobiaceae* with validly published names were more distantly related, showing sequence similarities of less than 94 %.

The DNA–DNA hybridization value between strains YM23-227^T and 06SJR1-1^T was around 8 %. This result strongly suggests that strains YM23-227^T and 06SJR1-1^T should be classified as representing two separate species (Wayne *et al.*, 1987).

Cells of strains YM23-227^T, 06SJR1-1^T, AK18-024^T and 05IJR53-1^T grown on 1/5-strength MA and of strains MN1-1037^T and MN1-1047 cultivated on MA were rod-shaped, mostly ranging around 1.0 µm in width and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of the new taxa described in this study among the currently known and cultivated species of the family *Verrucomicrobiaceae*. Numbers at nodes are bootstrap percentages derived from 1000 replications. GenBank/EMBL/DDBJ accession numbers are given in parentheses. Sequences determined in this study are shown in bold. The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 2% sequence divergence.

1.5 µm in length. Cells of these six strains divided by means of binary fission; the cells were non-motile and flagella and appendages were not observed.

Analysis of the pigments produced by the strains was performed using HPLC/PAD (photodiode array detection)/APCI (atmospheric pressure chemical ionization)–MS (mass spectrometry) (TermoFinnigan) of the crude acetone extract from frozen cells. These pigments showed UV-VIS spectra with absorption maxima at 446 and 472 nm (strain YM23-227^T), 494 and 524 nm (strain 06SJR1-1^T), 460, 493 and 525 nm and 459 and 488 nm (strain AK18-024^T, containing two types of pigment), 446 and 472 nm (strain 05IJR53-1^T) and 450 and 478 nm (strains MN1-1037^T and MN1-1047), characteristic of carotenoids. The chemical structures of the detected carotenoids have yet to be determined. Squalene was

detected in all strains examined using the HPLC/PAD/APCI-MS system.

As shown in Table 2, the predominant cellular fatty acid of strains YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047 was iso-C_{14:0} (35.7–49.9%), which distinguishes them from cultivated members of the family *Verrucomicrobiaceae*. Also, based on the fatty acid profiles, these strains could be separated into an independent group.

The novel isolates could be distinguished from other taxa of the class *Verrucomicrobiae* by the phenotypic characteristics given in Table 3. The cell walls of all the novel isolates were prepared by disruption of cells, followed by heating with 3% SDS, washing and centrifugation. Amino acid analysis of the cell wall hydrolysates indicated the presence

Table 2. Comparison of cellular fatty acid contents (%) of the novel strains and related taxa of the class *Verrucomicrobiae*

Taxa: 1, strain YM23-227^T (*H. harenae* sp. nov.); 2, strain 06SJR1-1^T (*H. rosea* sp. nov.); 3, strain AK18-024^T (*H. phyci* sp. nov.); 4, strain 05IJR53-1^T (*H. helveola* sp. nov.); 5, strain MN1-1037^T (*H. sargassicola* sp. nov.); 6, strain MN1-1047; 7, *Persicirhabdus* spp. (data from Yoon *et al.*, 2008b); 8, *Roseibacillus* spp. (Yoon *et al.*, 2008b); 9, *Luteolibacter* spp. (Yoon *et al.*, 2008b); 10, *Rubritalea* spp. (Scheuermayer *et al.*, 2006; Kasai *et al.*, 2007; Yoon *et al.*, 2007, 2008a). Data are expressed as percentages of total fatty acids. Fatty acids representing less than 1 % are not shown. —, Not detected; tr, trace.

Fatty acid	1	2	3	4	5	6	7	8	9	10
Saturated										
C _{13:0}	—	—	tr	tr	—	—	—	—	tr	—
C _{14:0}	tr	tr	tr	1	5.3	5.9	9.7–11.7	tr–5.5	4.4–13.9	tr–2.9
C _{15:0}	6	2.6	13.2	7.8	4.6	3.1	1.1	1.4–5	1.9–3.4	1–4.5
C _{16:0}	6.1	2.5	11.6	9.2	22.3	27.6	54.7–59.6	18.6–38.6	15.6–20.2	2.5–23.1
C _{17:0}	tr	tr	8.7	tr	tr	—	—	1.9–6.3	tr	tr–2.8
C _{18:0}	—	—	tr	tr	1.3	1.4	1.9	tr–2.4	1.2–1.3	tr–2.4
Unsaturated										
C _{14:1} ω5c	—	—	—	—	—	—	—	—	1.6–3	—
C _{14:1} ω7c	—	—	—	—	—	—	4.2–5.5	—	—	—
C _{15:1} ω6c	—	—	—	—	—	—	—	—	—	tr–2.1
C _{16:1} ω7c	—	tr	tr	tr	2.6	3.2	22.1–23.2	1.1–1.4	6.7–9.9	7–21.5
C _{16:1} ω9c	1.7	3.3	4.7	4.7	5.3	6	—	12.2–25.2	—	—
C _{17:1} ω8c	—	—	—	—	—	—	—	tr–1.2	—	—
C _{18:1} ω7c	—	—	—	—	—	—	—	—	—	1.5
C _{18:1} ω9c	—	—	tr	tr	tr	tr	—	tr–1.8	—	—
Branched										
iso-C _{14:0}	41.2	49.9	37.6	47.1	39.3	35.7	2.7	16.5–37.5	43–45.4	22–49.4
iso-C _{15:0}	tr	1.3	tr	2.5	—	—	—	—	—	—
iso-C _{16:0}	22.3	18.7	8.8	8.5	5.8	6.5	tr	2–9.7	2.3–14.8	12.2–29.1
iso-C _{18:0}	—	—	—	—	—	—	—	tr–1.6	—	—
anteiso-C _{15:0}	20.3	17.5	11.3	15.3	9.1	8	1.6	1.1–2.3	3.7–10.5	4.7–18.1
anteiso-C _{17:0}	tr	tr	tr	tr	—	—	—	—	tr	1.3–2
Unidentified										
C _{16:1} /cyclo C _{16:0}	—	—	—	—	—	—	—	11	—	—

of muramic acid and *meso*-diaminopimelic acid in the cell wall peptidoglycan of the novel isolates.

Based on the results of the phylogenetic analysis and their biochemical and physiological properties, the novel six strains, YM23-227^T, 06SJR1-1^T, AK18-024^T, 05IJR53-1^T, MN1-1037^T and MN1-1047, isolated from various marine environments represent a new genus and five novel species of the family *Verrucomicrobiaceae* within the phylum 'Verrucomicrobia'. The names *Haloferula rosea* gen. nov., sp. nov., *Haloferula harenae* sp. nov., *Haloferula phyci* sp. nov., *Haloferula helveola* sp. nov. and *Haloferula sargassicola* sp. nov. are proposed.

Description of *Haloferula* gen. nov.

Haloferula (Ha.lo.fe'ru.la. Gr. n. *hals halos*, sea; L. fem. n. *ferula* a stick, cane; N.L. fem. n. *Haloferula*, a rod-shaped bacterium from the sea).

Cells are rod-shaped, Gram-negative and obligately aerobic. Cells lack flagella and are non-motile. Non-spore-forming. Chemoheterotrophs. Nitrate is not reduced. The major respiratory quinone is MK-9. The G + C content of

the genomic DNA is 63–65 mol%. Predominant cellular fatty acid is iso-C_{14:0}. The type species is *Haloferula rosea*.

Description of *Haloferula rosea* sp. nov.

Haloferula rosea (ro.se'a. L. fem. adj. *rosea* rose-coloured, rosy).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.8–1.0 µm in width and 1.0–1.5 µm in length. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA are circular, convex and pale-pink in colour. Temperature range for growth is 20–37 °C. Optimal temperature for growth is 25–30 °C. No growth occurs at either 4 or 45 °C. pH range for growth is 6.5–9.0. NaCl is required for growth and can be tolerated up to 5 % (w/v). Catalase-negative but oxidase-positive. Alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive, but esterase (C4) and *N*-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, valine arylamidase, trypsin, esterase lipase (C8), lipase (C4), cystine arylamidase, chymotrypsin,

Table 3. Phenotypic characteristics of novel strains belonging to *Haloferula* gen. nov., compared with related taxa of the class *Verrucomicrobiae*

Taxa: 1, strain YM23-227^T (*H. harenae* sp. nov.); 2, strain 06SJR1-1^T (*H. rosea* sp. nov.); 3, strain AK18-024^T (*H. phyci* sp. nov.); 4, strain 05IJR53-1^T (*H. helveola* sp. nov.); 5, strain MN1-1037^T (*H. sargassicola* sp. nov.); 6, strain MN1-1047; 7, *Persicirhabdus sediminis* YM20-087^T (data from Yoon *et al.*, 2008b); 8, *Roseibacillus ishigakijimensis* MN1-741^T (Yoon *et al.*, 2008b); 9, *Roseibacillus ponti* YM27-120^T (Yoon *et al.*, 2008b); 10, *Roseibacillus persicus* YM26-010^T (Yoon *et al.*, 2008b); 11, *Luteolibacter pohnpeiensis* A4T-83^T (Yoon *et al.*, 2008b); 12, *Luteolibacter algae* A5J-41-2^T (Yoon *et al.*, 2008b); 13, *Prostheco bacter fusiformis* FC4^T (Staley *et al.*, 1976; Hedlund *et al.*, 1997b); 14, *Verrucomicrobium spinosum* DSM 4136^T (Schlesner, 1987); 15, *Akkermansia muciniphila* Muc^T (Derrien *et al.*, 2004); 16, *Rubritalea marina* Pol012^T (Scheuermayer *et al.*, 2006). +, Positive; w, weakly positive; −, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Isolation source	Sediment	Sponge	Algae	Seawater	Algae	Algae	Sediment	Seawater	Brown alga	Iron sand	Driftwood piece	Red algae	Marl pond	Lake	Human faeces	Sponge
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Fusiform rod	Fusiform rod	Oval- shaped	Coccoid or rod
Colony colour	Pink	Pale- pink	Pale- orange	Yellow	Pale- orange	Pale- orange	Pale-pink	Pale-pink	Reddish pink	Pink	Pale-yellow	Pale- yellow	Opaque- yellow	Light- yellow	White	Red
Tolerance to oxygen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Prosthecae	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
Fimbriae	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−
Production of:																
Catalase	w	−	−	−	+	+	−	+	w	w	+	+	+	+	ND	−
Oxidase	+	+	+	+	−	−	+	+	+	+	+	+	−	+	ND	+
Temperature range (°C)	20–37	20–37	20–37	20–37	4–37	4–37	20–30	20–37	15–37	15–37	20–37	4–37	4–40	26–34	20–40	8–30
Growth at 4 °C	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	−
Growth at 37 °C	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	−
pH range	6–9	6.5–9	6–9	6–8.5	6.5–9	6.5–9	6.5–8.5	6.5–9	7–9	6.5–9	6.5–9	6.5–8.5	ND	9	5.5–8	6.8–8.2
NaCl tolerance (%)	5	5	5	6	7	7	2.5	6	7	7	6	3	ND	1	ND	3.8
NaCl requirement	+	+	+	+	+	+	−	+	+	+	+	−	ND	−	ND	+
DNA G+C content (mol%)	63.8	63.1	64.3	64.9	64.7	64.8	50.4	61.4	57.6	58.3	55.8	55.9	56.1	58.6	47.6	50.9
Major menaquinone	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9, MK-10	MK-9	ND	ND	ND	MK-8, MK-9

β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase are negative. Dextrin, glycogen, *N*-acetyl-D-galactosamine, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, lactulose, maltose, D-mannose, melibiose, raffinose, sucrose, trehalose, furanose, succinic acid monomethyl ester, D-galacturonic acid, succinamic acid and bromosuccinic acid are oxidized, but Tween 80, DL-lactic acid, L-fucose, *cis*-aconitic acid, citric acid, succinic acid, glucose 1-phosphate, glucose 6-phosphate, cyclodextrin, Tween 40, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, i-erythritol, *myo*-inositol, D-mannitol, methyl β -D-glucoside, D-psicose, L-rhamnose, D-sorbitol, xylitol, pyruvic acid methyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL- α -glycerol phosphate are not oxidized. Cell wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{15:0}, C_{16:0}, C_{16:1} ω 9c, iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G+C content of the type strain is 63.1 mol%.

The type strain, 06SJR1-1^T (=MBIC08340^T=KCTC 22201^T), was isolated from a marine sponge.

Description of *Haloferula harenae* sp. nov.

Haloferula harenae (ha.re.na'e. L. gen. n. *harenae* from sand).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.8–1.0 μ m in width and 1.5–2.0 μ m in length. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA are circular, convex and pink in colour. Temperature range for growth is 20–37 °C. Optimal temperature for growth is 25–30 °C. No growth occurs at either 4 or 45 °C. pH range for growth is 6.0–9.0. NaCl is required for growth and can be tolerated up to 5% (w/v). Catalase- and oxidase-positive. Alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase and *N*-acetyl- β -glucosaminidase are positive, but esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase are negative. Dextrin, *N*-acetyl-D-glucosamine, L-

arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, lactulose, maltose, D-mannose, melibiose, D-psicose, raffinose, sucrose, trehalose, furanose, D-galacturonic acid, succinic acid and glycerol are oxidized, but Tween 80, glucose 6-phosphate, L-fucose, D-mannitol, D-sorbitol, acetic acid, *cis*-aconitic acid, α -ketobutyric acid, α -ketoglutaric acid, inosine, glucose 1-phosphate, cyclodextrin, glycogen, Tween 40, *N*-acetyl-D-galactosamine, adonitol, D-arabitol, i-erythritol, *myo*-inositol, methyl β -D-glucoside, L-rhamnose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ -amino butyric acid, urocanic acid, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol and DL- α -glycerol phosphate are not oxidized. Cell wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{15:0}, C_{16:0}, C_{16:1} ω 9c, iso-C_{14:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G+C content of the type strain is 63.8 mol%.

The type strain, is YM23-227^T (=MBIC08299^T=KCTC 22198^T), was isolated from marine sediment.

Description of *Haloferula phyci* sp. nov.

Haloferula phyci (phy'ci. L. gen. n. *phyci* of a sea-weed).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.8–1.0 μ m in width and 1.2–1.5 μ m in length. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA medium are circular, convex and pale-orange in colour. Temperature range for growth is 20–37 °C. Optimal temperature for growth is 25–30 °C. No growth occurs at either 4 or 45 °C. pH range for growth is 6.0–9.0. NaCl is required for growth and can be tolerated up to 5% (w/v). Catalase-negative but oxidase-positive. Alkaline phosphatase, acid phosphatase and β -galactosidase are positive, but esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, esterase (C4), leucine arylamidase, *N*-acetyl- β -glucosaminidase, α -galactosidase, α -glucosidase, valine arylamidase, trypsin, lipase (C4), cystine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase are negative. L-Arabinose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, melibiose, D-psicose, raffinose, acetic acid, α -ketobutyric acid, α -ketoglutaric acid, succinic acid, succinamic acid, L-ornithine, inosine and DL- α -glycerol phosphate are

oxidized, but dextrin, lactulose, D-mannitol, D-mannose, furanose, D-galacturonic acid, DL-carnitine, γ -aminobutyric acid, uridine, thymidine, 2,3-butanediol, glycerol, L-fucose, *cis*-aconitic acid, citric acid, glucose 1-phosphate, glucose 6-phosphate, cyclodextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, i-erythritol, gentiobiose, *myo*-inositol, methyl β -D-glucoside, L-rhamnose, D-sorbitol, sucrose, trehalose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, urocanic acid, phenylethylamine, putrescine and 2-aminoethanol are not oxidized. Cell wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{15:0}, C_{16:0}, C_{17:0}, C_{16:1} ω 9c, iso-C_{14:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G+C content of the type strain is 64.3 mol%.

The type strain, AK18-024^T (=MBIC08341^T=KCTC 22200^T), was isolated from marine algae.

Description of *Haloferula helveola* sp. nov.

Haloferula helveola (hel.ve'ol.a. L. fem. adj. *helveola* pale yellow, yellowish).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.8–1.0 μ m in width and 1.0–1.2 μ m in length. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA medium are circular, convex and yellow in colour. Temperature range for growth is 20–37 °C. Optimal temperature for growth is 25–30 °C. No growth occurs at either 4 or 45 °C. pH range for growth is 6.0–8.5. NaCl is required for growth and can be tolerated up to 6% (w/v). Catalase-negative but oxidase-positive. Alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive, but leucine arylamidase, trypsin, α -glucosidase, esterase lipase (C8), esterase (C4), *N*-acetyl- β -glucosaminidase, α -galactosidase, β -galactosidase, valine arylamidase, lipase (C4), cystine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase are negative. Dextrin, cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, lactulose, maltose, D-mannose, melibiose, raffinose, sucrose, trehalose and furanose are oxidized, but glycogen, L-arabinose, xylitol, succinic acid, L-glutamic acid, DL-carnitine, urocanic acid, DL- α -glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate, L-fucose, *cis*-aconitic acid, citric

acid, cyclodextrin, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, i-erythritol, gentiobiose, *myo*-inositol, D-mannitol, methyl β -D-glucoside, D-psicose, L-rhamnose, D-sorbitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, γ -aminobutyric acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol and glycerol are not oxidized. Cell wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{14:0}, C_{15:0}, C_{16:0}, C_{16:1} ω 9c, iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G+C content of the type strain is 64.9 mol%.

The type strain, 05IJR53-1^T (=MBIC08342^T=KCTC 22199^T), was isolated from seawater.

Description of *Haloferula sargassicola* sp. nov.

Haloferula sargassicola (sar.gas'si.co'la. L. n. *Sargassum* a genus name of planktonic macroalgae; L. suff. *-cola* from L. n. *incola* inhabitant; N.L. n. *sargassicola* inhabitant of *Sargassum*).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.8–1.0 μ m in width and 1.0–1.5 μ m in length. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on MA medium are circular, convex and pale-orange in colour. Temperature range for growth is 4–37 °C. Optimal temperature for growth is 20–30 °C. No growth occurs at 45 °C. pH range for growth is 6.5–9.0. NaCl is required for growth and can be tolerated up to 7% (w/v). Catalase-positive but oxidase-negative. Alkaline phosphatase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -mannosidase are positive, but esterase lipase (C8), leucine arylamidase, valine arylamidase, α -galactosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase, α -glucosidase, esterase (C4), lipase (C4), cystine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase and α -fucosidase are negative. Dextrin, glycogen, *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, lactulose, maltose, D-mannose, melibiose, methyl β -D-glucoside, sucrose, trehalose, furanose, pyruvic acid methyl ester and thymidine are oxidized, but i-erythritol, D-psicose, raffinose, D-sorbitol, glycerol, glucose 6-phosphate, L-fucose, *cis*-aconitic acid, citric acid,

succinic acid, cyclodextrin, Tween 40, Tween 80, N-acetyl-D-galactosamine, adonitol, D-arabitol, myo-inositol, D-mannitol, L-rhamnose, xylitol, succinic acid monomethyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglytamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL- α -glycerol phosphate and glucose 1-phosphate are not oxidized. Cell wall peptidoglycan contains muramic acid and meso-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{14:0}, C_{15:0}, C_{16:0}, C_{18:0}, C_{16:1} ω 7c, C_{16:1} ω 9c, iso-C_{14:0}, iso-C_{16:0} and anteiso-C_{15:0}. The DNA G + C content is 64.7–64.8 mol%.

The type strain, MN1-1037^T (=MBIC08343^T=KCTC 22202^T), and strain MN1-1047 were isolated from marine algae (*Sargassum*).

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