Geofilum rubicundum gen. nov., sp. nov., isolated from deep seafloor sediment

Masayuki Miyazaki,¹ Osamu Koide,² Tohru Kobayashi,² Kozue Mori,³ Shigeru Shimamura,¹ Takuro Nunoura,¹ Hiroyuki Imachi,¹ Fumio Inagaki,⁴ Takahiko Nagahama,² Yuichi Nogi,² Shigeru Deguchi² and Ken Takai¹

Correspondence

Masayuki Miyazaki
miyazakim@jamstec.go.jp

¹Subsurface Geobiology Advanced Research (SUGAR) project, Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

²Soft Matter and Extremophiles Research Team, Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

³Marine Bioresource Exploration Research Team and Institute of Biogeosciences (BioGeos), Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

⁴Geomicrobiology Group, Kochi Institute for Core Sample Research, Japan Agency for Marine–Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan

A novel, facultatively anaerobic bacterium (strain JAM-BA0501^T) was isolated from a deep seafloor sediment sample at a depth of 247 m below seafloor off the Shimokita Peninsula of Japan in the north-western Pacific Ocean (Site C9001, 1180 m water depth). Cells of strain JAM-BA0501^T were Gram-negative, filamentous, non-spore-forming and motile on solid medium by gliding. Phylogenetic analysis based on the 16S rRNA gene sequence of strain JAM-BA0501^T indicated a distant relationship to strains representing genera within the order *Bacteroidales*, such as *Alkaliflexus imshenetskii* Z-7010^T (91.1 % similarity), *Marinilabilia salmonicolor* ATCC 19041^T (86.2 %) and *Anaerophaga thermohalophila* Fru22^T (89.3 %). The new isolate produced isoprenoid quinones with menaquinone MK-7 as the major component, and the predominant fatty acids were iso-C_{15:0} and anteiso-C_{15:0}. The DNA G+C content of the isolate was 42.9 mol%. Based on its taxonomic distinctiveness, strain JAM-BA0501^T is considered to represent a novel species of a new genus within the family *Marinilabiliaceae*, for which the name *Geofilum rubicundum* gen. nov., sp. nov. is proposed. The type strain of *Geofilum rubicundum* is JAM-BA0501^T (=JCM 15548^T =NCIMB 14482^T).

The family *Marinilabiliaceae* within the order *Bacteroidales* was proposed by Ludwig *et al.* (2008). At the time of writing, this family includes three genera, namely *Marinilabilia*, *Alkaliflexus* and *Anaerophaga*. *Marinilabilia salmonicolor* was originally described as a species of the genus *Cytophaga* by Veldkamp (1961) to accommodate a novel, facultatively anaerobic, agar-degrading bacterium isolated from marine sediment. Based on phylogenetic affiliation as well as physiological and chemotaxonomic characteristics, this bacterium was transferred to a novel genus, *Marinilabilia*, by Nakagawa & Yamasato (1996). The

genus *Alkaliflexus* was described and proposed by Zhilina *et al.* (2004) for a novel, anaerobic, low-O₂-tolerant, alkaliphilic bacterium isolated from soda lake sediments. The genus *Anaerophaga* was erected to accommodate a novel, strictly anaerobic, thermophilic bacterium isolated from an oilfield (Denger *et al.*, 2002). All recognized members of the family are facultatively or strictly anaerobic heterotrophs.

A diversity of aerobic heterotrophic bacteria were isolated from seafloor sediment samples cored from offshore of the Shimokita Peninsula, Japan, by the deep-earth research drilling vessel *Chikyu* in 2006 from multiple sample depths down to 365 m below the seafloor (drilling site C9001C, 41° 10.6380' N 142° 12.081' E: see Kobayashi *et al.*, 2008). The depth of overlying seawater at Site C9001C is 1180 m. Preliminary phylogenetic analysis based on 16S rRNA gene sequences showed that several isolates might represent

Abbreviations: ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JAM-BA0501^T is AB362265.

One supplementary table and three supplementary figures are available with the online version of this paper.

novel phylotypes within the order *Bacteroidales* (Kobayashi *et al.*, 2008).

In this study, we characterized the taxonomic properties of one of these strains, designated JAM-BA0501^T, isolated from a deep sediment core sample at 247 m below the seafloor, which appeared to represent a novel, facultatively anaerobic member of a new genus within the family *Marinilabiliaceae*. Strain JAM-BA0501^T was purified by colony-isolation on marine agar 2216 (MA; Difco) at 30 °C. The isolate was maintained on MA plates or in marine broth 2216 (MB; Difco) at 30 °C and stored at –80 °C in 15 % (v/v) glycerol.

Cells were observed routinely by using an Olympus BX51 microscope. Transmission electron microscopy of negatively stained cells and sections of cells was conducted with a JEM-1210 (JEOL) as described by Nogi *et al.* (1998). Cells of strain JAM-BA0501^T in the mid-exponential phase of growth in MA at 30 °C were used for electron microscopic observations. Cells were filamentous, approx. 0.2–0.4 µm wide and 4.0–22.0 µm long (Fig. 1) and with both outer and inner membrane structures (Fig. 1b). Many fimbriae-like structures were observed on the cell surface (Fig. 1a). Strain JAM-BA0501^T was motile on solid medium by means of gliding.

Unless otherwise noted, physiological properties of strain JAM-BA0501^T were examined by using YPA medium containing 0.5 % (w/v) yeast extract (Difco) and 0.5 % (w/v) peptone (Difco) based on artificial seawater (ASW: consisting of 2.75 % NaCl, 0.07 % KCl, 0.54 % MgCl₂ · 6H₂O, 0.68 % MgSO₄ · 7H₂O, 0.14 % CaCl₂ · 2H₂O and 0.02 % NaHCO₃), modified slightly from that given by Nogi & Kato (1999), or MB. The effect of temperature, NaCl concentration and pH on cell growth was determined by examining the time course of optical density (temperature gradient incubator with a bio-photorecorder, model TVS126MA; Advantec). Growth at 10–45 °C was tested in MB; the optimal growth temperature was 33 °C. Cell growth was observed at 10 and 35 °C, but not above 38 °C (see Fig. S1a available in IJSEM Online). The effect of NaCl concentration on growth was examined with YPA medium in the presence of 0–7 % (w/v) NaCl. Optimal growth occurred with 1 % NaCl. Cell growth was observed in the

presence of 0.5 and 6 % NaCl, but not in the absence of NaCl or in the presence of >7 % NaCl (Fig. S1b). The optimal pH and pH range for cell growth were determined in YPA buffered with 10 mM Tris to a pH of 6.4, 6.9, 7.3, 7.8, 8.3, 8.8, 9.3 or 9.8 at room temperature (approx. 25 °C). Optimal growth occurred at pH 7.3–8.3. Cell growth was observed at pH 6.9 and 9.3, but not at pH 6.4 or at greater than pH 9.8 (Fig. S1c).

To determine the utilization of organic substrates for cell growth, each of the following substrates was added to ASW supplemented with 0.1 % (w/v) NH₄Cl and 10 mM Tris (pH 8.0) in the presence or absence of 0.01 % (w/v) yeast extract: L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-sorbitol, L-sorbose, sucrose, trehalose, xylose (all at 1.0 %, w/v), L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine or L-valine (all at 0.5 %, w/v). We also tested cell growth in ASW containing 1.0, 0.1 or 0.01 % (w/v) yeast extract as the sole substrate. The isolate was able to grow in ASW containing 1.0 and 0.1 % (w/v) yeast extract solely, but no cell growth was observed in ASW medium containing 0.01 % (w/v) yeast extract. In addition, strain JAM-BA0501^T did not grow on the sugars and amino acids that we tested as sole energy and carbon sources. However, when these substrates were supplemented with ASW containing 0.01 % (w/v) yeast extract, the isolate was able to grow with cellobiose, D-fructose, D-galactose, lactose, D-mannose, L-rhamnose, trehalose and xylose. These results indicated that strain JAM-BA0501^T utilized such sugars as energy and carbon source.

Oxidase activity was determined by spreading cell pellets on oxidase test paper (Nissui Pharmaceutical). Catalase activity was determined based on O₂-bubble production in 3 % (v/v) H₂O₂ solution. Agarase, amylase, cellulase, chitinase, gelatinase, lipase (tri-*n*-butyrin), protease and xylanase activities were tested on MA plates by using each substrate at 1 % (w/v). DNase activity was assessed with DNase Test Agar (Difco). Hydrolysis of aesculin, ONPG,

Fig. 1. Transmission electron micrograph of a negatively stained cell (a) and thin section of a cell (b) of strain JAM-BA0501^T. Fimbriae-like structures are indicated by arrows (a). Bars, 1.0 µm (a) and 100 nm (b).

and Tweens 20, 40 and 80 was studied as described by Barrow & Feltham (1993). Based on these enzyme assays of strain JAM-BA0501^T, the isolate was found to be positive only for amylase and protease activities and was able to hydrolyse aesculin, ONPG, Tween 20 and Tween 40.

Antibiotic susceptibility was tested in MB. Strain JAM-BA0501^T was susceptible to (per millilitre) ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin (10 µU), rifampicin (5 µg), tetracycline (30 µg) and vancomycin (30 µg), but resistant to gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg) and neomycin (30 µg).

Nitrate and nitrite reduction were determined as described by Barrow & Feltham (1993). Hydrogen sulfide production from thiosulfate and the production of indole were assessed by using sulfide indole motility agar (Nissui Pharmaceutical) slabs prepared with ASW instead of water. Strain JAM-BA0501^T reduced nitrate and nitrite, but did not produce hydrogen sulfide or indole.

Anaerobic growth of strain JAM-BA0501^T was tested with YPA medium in the presence or absence of 0.1 % (w/v) KNO₃ under a 100 % N₂ atmosphere (100 kPa). The isolate grew with YPA medium in the presence of nitrate but did not show fermentative growth. This indicated that strain JAM-BA0501^T was a facultative anaerobe, growing via O₂-respiration and nitrate-reduction under aerobic and anaerobic conditions, respectively. The general physiological characteristics of strain JAM-BA0501^T are summarized in Table 1 or are given in the genus and species descriptions below.

The absorption spectrum of pigment extracted with 3 ml acetone/methanol (7:2) (v/v) from harvested cells (1 g wet pellet) was determined at 300–700 nm with a Hitachi U-2000 spectrophotometer (Miyazaki *et al.*, 2010). Cells of strain JAM-BA0501^T produced salmon pink pigments with maximum absorbance at 480 nm (Table 1).

Cells of strain JAM-BA0501^T at the late-exponential phase of growth in MB at 30 °C were used for characterization of isoprenoid quinones, cellular fatty acids and polar lipids. Isoprenoid quinones were extracted with chloroform/methanol (2:1) (v/v) from lyophilized cells (200 mg) and purified by TLC. The purified isoprenoid quinones were analysed by using HPLC (Komagata & Suzuki, 1987). The major isoprenoid quinone of strain JAM-BA0501^T was menaquinone MK-7. The fatty acids of strain JAM-BA0501^T and related genera were obtained from cells by saponification, methylation and extraction according to the Sherlock Microbial Identification System (MIDI, 1999). Fatty acid compositions were determined by using a Finnigan TRACE DSQ GC-MS system (Thermo Fisher Scientific) equipped with a DB-5 column (J&W Scientific) under a helium flow of 1.5 ml min⁻¹ and an oven temperature programme increasing from 140 °C (5 min) to 280 °C (5 min) at 4 °C min⁻¹. The major fatty acids were iso-C_{15:0} and anteiso-C_{15:0}

(Table S1). The polar lipids of strain JAM-BA0501^T were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by using two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). Polar lipid analysis revealed the presence of phosphatidylethanolamine, diphosphatidylglycerol and an unknown glycolipid (Fig. S2).

Genomic DNA of the isolate was extracted and purified by using the phenol extraction method (Saito & Miura, 1963). The DNA G + C content was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). The almost full-length 16S rRNA gene sequence (~1440 bp) of strain JAM-BA0501^T was determined by direct sequencing of PCR-amplified DNA as described previously (Miyazaki *et al.*, 2008). The sequence was aligned with a subset of 16S rRNA gene sequences obtained from public databases via the FASTAligner utility of ARB software (Ludwig *et al.*, 2004). Phylogenetic trees were reconstructed with the neighbour-joining (NJ) and maximum-likelihood (ML) methods by using PAUP 4.0 beta 10 (Swofford, 1998). The NJ tree (Saitou & Nei, 1987) was reconstructed by using the parameters of the Jukes–Cantor model distance. The ML tree (Felsenstein, 1981) was drawn by using the default starting parameters (NJ with a Jukes–Cantor model of evolution). Bootstrap confidence of branching was calculated based on 1000 and 250 replications for the NJ and ML trees, respectively.

The phylogenetic tree inferred from the NJ method is shown in Fig. 2 (a global tree is available as Fig. S3). The result of phylogenetic analysis indicated that strain JAM-BA0501^T was affiliated to the family *Marinilabiliaceae* within the order *Bacteroidales* and was distantly related to *Alkaliflexus imshenetskii* Z-7010^T (91.1 % 16S rRNA gene sequence similarity), *Marinilabilia salmonicolor* ATCC 19041^T (86.2 %) and *Anaerophaga thermohalophila* Fru22^T (89.3 %).

Although strain JAM-BA0501^T had morphological and physiological properties similar to those of members of the order *Bacteroidales*, several taxonomic features, such as non-spherical cell formation and major fatty acid composition, differ from those of related genera in the family *Marinilabiliaceae* and even other genera within the order *Bacteroidales* (Table 1). In addition, the NJ phylogenetic tree indicates that strain JAM-BA0501^T is related most closely to *Alkaliflexus imshenetskii* Z-7010^T (Fig. 2). Although the two strains share many taxonomic properties (Table 1), *Alkaliflexus imshenetskii* Z-7010^T is not able to grow aerobically or to reduce nitrate (Table 1). They also differ in utilization pattern of carbohydrates and 3-hydroxy fatty acid composition. On the basis of these phenotypic and phylogenetic characteristics, strain JAM-BA0501^T is considered to represent a novel species of a new genus within the family *Marinilabiliaceae*, for which we propose the name *Geofilum rubicundum* gen. nov., sp. nov.

Table 1. Differential characteristics between strain JAM-BA0501^T and related type species of the family *Marinilabiliaceae*

Strains: 1, JAM-BA0501^T; 2, *Alkaliflexus imshenetskii* Z-7010^T; 3, *Marinilabilia salmonicolor* ATCC 19041^T; 4, *Anaerophaga thermohalophila* Fru22^T; 5, *Cytophaga fermentans* ATCC 19072^T; 6, *Marinifilum fragile* JC2469^T. Data for reference type strains are from Bachmann (1955), Denger *et al.* (2002), Holt *et al.* (1994), Na *et al.* (2009), Suzuki *et al.* (1999), Veldkamp (1961) and Zhilina *et al.* (2004) except where indicated otherwise. ND, No data.

Characteristic	1	2	3	4	5	6
Origin	Subsurface	Soda lake	Marine	Oilfield	Marine	Marine
Colony colour	Salmon pink	Pink	Yellow to salmon pink	Orange-red	Bright yellow	Ivory or brownish ivory
Cell form						
Size	0.2–0.4 × 4.0–22.0	0.25–0.4 × 4–10	0.3–0.5 × 2–50	0.3 × 4–8	0.5–0.7 × 2–10	0.5 × 0.3–3.8
Motility	Gliding	Gliding	Gliding	Not observed	Gliding	Flagella-like
Coccoid forms	–	+	+	–	ND	ND
Growth (°C, after 1 week)						
Optimum	33	35	28–37	55	30	33.8
Range	10–36	10–45	ND	37–55	ND	20–37.4
NaCl (%; 1 week)						
Optimum	1.0	2.0	ND	2.0–6.0	ND	3
Range	0.5–6.0	0.08–5.3	1.0–3.0	2.0–12.0	<3.0	1–7
pH						
Optimum	7.3–8.3	8.5	7.0–7.5	6.8	ND	7
Range	6.9–9.3	7.2–10.2	ND	ND	ND	6–8
O ₂ metabolism	Facultatively anaerobic	Anaerobic; low O ₂ tolerance	Facultatively anaerobic	Strictly anaerobic	Facultatively anaerobic	Facultatively anaerobic
Catalase	+	+	+	–	+	–
Oxidase	–	ND	ND	–	ND	+
Agarase	–	–	ND	–	+	ND
Gelatinase	+	–	+	ND	ND	+
Xylanase	–	+	+	+	+	ND
Nitrate reduction	+	–	+	ND	–	+
Major fatty acid(s)†	i15:0, a15:0	i15:0, a15:0*	i13:0, i15:0, a15:0*	i15:0, a15:0	ND	i15:0
3-Hydroxylated cellular fatty acids	i15:0, a15:0, 16:0, i16:0, i17:0, a17:0	i15:0, 16:0, i17:0, a17:0*	i15:0, a15:0, i16:0, i17:0*	15:0, 16:0, i16:0, i17:0	ND	i15:0, i17:0
Absorption maximum(a) of pigments (nm)	480	485	ND	488, 518	ND	ND
DNA G + C content (mol%)	42.9	44.3	37	41.8	39	45

*Data from the present study.

†i, Iso branched; a, anteiso branched.

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of representative genera and species within the family *Marinilabiliaceae*. The tree was rooted with *Bacteroides fragilis* ATCC 25285^T. Bootstrap values (percentages of 1000 replicates) are given at branch points; only values >50% are shown. Solid circles indicate that the corresponding nodes (groups) were also recovered by using the ML method at >50% support (250 replicates). GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 0.02 nt substitutions per site. An expanded version of this tree is available as Fig. S3.

Description of *Geofilum* gen. nov.

Geophilum (Ge.o.fi'lum. Gr. n. *ge* the earth; L. neut. n. *filum* a thread; N.L. neut. n. *Geophilum* a thread from the earth).

Cells are Gram-negative, filamentous and non-spore-forming. Facultatively anaerobic and organoheterotrophic. Catalase-positive. Motile by means of gliding. Mesophilic. NaCl is required for growth. The major respiratory quinone is MK-7. The cellular fatty acid profile is dominated by branched saturated components. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol and an unknown glycolipid. The genus is affiliated to the family *Marinilabiliaceae*. The type species is *Geofilum rubicundum*.

Description of *Geofilum rubicundum* sp. nov.

Geofilum rubicundum (ru.bi.cun'dum. L. neut. adj. *rubicundum* red, ruddy).

Displays the following properties in addition to those given in the genus description. Cell width ranges from 0.2 to 0.4 µm, and cell length ranges from 4.0 to 22.0 µm. Cells possess numerous fimbriae but no flagellum. Colonies on solid medium are circular with entire edges, smooth, convex and salmon pink, 0.5–1.0 mm in diameter after 3–4 days of incubation at 30 °C. The optimal temperature for growth is 33 °C. Growth occurs at 10 and 36 °C, but not above 38 °C. Optimal growth occurs in the presence of 1% NaCl. Growth occurs in the presence of 0.5 and 6% NaCl, but not without NaCl or in the presence of >7% NaCl. The optimal pH for growth is 7.3–8.3. Growth occurs at pH 6.9 and 9.3, but not at pH 6.4 or above pH 9.8. Cytochrome oxidase-negative. Does not produce hydrogen sulfide or indole. Nitrate and nitrite are reduced. Positive for amylase and gelatinase. Negative for agarase, cellulase, chitinase, DNase, lipase (tri-*n*-butyrin), protease and xylanase. Hydrolyses aesculin, ONPG, and Tweens 20 and 40, but not Tween 80. Utilizes cellobiose, D-fructose, D-galactose, lactose, D-mannose, L-rhamnose, trehalose and

xylose, but not L-arabinose, D-glucose, glycerol, myo-inositol, maltose, D-mannitol, raffinose, D-sorbitol, L-sorbose or sucrose. Susceptible to (per millilitre) ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin (10 µU), rifampicin (5 µg), tetracycline (30 µg) and vancomycin (30 µg), but tolerant of gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg) and neomycin (30 µg). The G + C content of the genomic DNA of the type strain is 42.9 mol% (determined by HPLC). The major isoprenoid quinone is MK-7. The dominant cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}.

The type strain, JAM-BA0501^T (=JCM 15548^T =NCIMB 14482^T), was isolated from a deep subseafloor sediment (247 m below the seafloor, 1180 m water depth) off the Shimokita Peninsula, Japan.

Acknowledgements

We thank Mr Katsuyuki Uematsu for assistance in preparing electron micrographs. We are very grateful to the shipboard scientists, technicians, drilling engineers and crew of the research vessel *Chikyu* for their help with sample collection.

References

- Bachmann, B. J. (1955). Studies on *Cytophaga fermentans*, n.sp., a facultatively anaerobic lower myxobacterium. *J Gen Microbiol* **13**, 541–551.
- Barrow, G. I. & Feltham, R. K. A. (editors) (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Denger, K., Warthmann, R., Ludwig, W. & Schink, B. (2002). *Anaerophaga thermohalophila* gen. nov., sp. nov., a moderately thermohalophilic, strictly anaerobic fermentative bacterium. *Int J Syst Evol Microbiol* **52**, 173–178.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors) (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams and Wilkins.

- Kobayashi, T., Koide, O., Mori, K., Shimamura, S., Matsuura, T., Miura, T., Takaki, Y., Morono, Y., Nunoura, T. & other authors (2008). Phylogenetic and enzymatic diversity of deep seafloor aerobic microorganisms in organics- and methane-rich sediments off Shimokita Peninsula. *Extremophiles* **12**, 519–527.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Ludwig, W., Euzéby, J. & Whitman, W. B. (2008) Draft Taxonomic Outline of the Bacteroidetes, Planctomycetes, Chlamydiae, Spirochaetes, Fibrobacteres, Fusobacteria, Acidobacteria, Verrucomicrobia, Dictyoglomi, and Gemmatimonadetes. Published online: http://www.bergeys.org/outlines/Bergeys_Vol_4_Outline.pdf
- MIDI (1999). *Sherlock, Microbial Identification System, Operating Manual, version 3.0*. Newark, DE: MIDI, Inc.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, K. & Parlett, J. H. (1984). An integrated procedure for extracting bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Miyazaki, M., Nogi, Y., Fujiwara, Y., Kawato, M., Kubokawa, K. & Horikoshi, K. (2008). *Neptunomonas japonica* sp. nov., an *Osedax japonicus* symbiont-like bacterium isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. *Int J Syst Evol Microbiol* **58**, 866–871.
- Miyazaki, M., Nagano, Y., Fujiwara, Y., Hatada, Y. & Nogi, Y. (2010). *Aquimarina macrocephali* sp. nov., isolated from sediment adjacent to sperm whale carcasses. *Int J Syst Evol Microbiol* **60**, 2298–2302.
- Na, H., Kim, S., Moon, E. Y. & Chun, J. (2009). *Marinifilum fragile* gen. nov., sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **59**, 2241–2246.
- Nakagawa, Y. & Yamasato, K. (1996). Emendation of the genus *Cytophaga* and transfer of *Cytophaga agarovorans* and *Cytophaga salmonicolor* to *Marinilabilia* gen. nov.: phylogenetic analysis of the *Flavobacterium*–*Cytophaga* complex. *Int J Syst Bacteriol* **46**, 599–603.
- Nogi, Y. & Kato, C. (1999). Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. *Extremophiles* **3**, 71–77.
- Nogi, Y., Kato, C. & Horikoshi, K. (1998). Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov. *Arch Microbiol* **170**, 331–338.
- Saito, H. & Miura, K. I. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (1999). Phylogenetic analysis of genus *Marinilabilia* and related bacteria based on the amino acid sequences of GyrB and emended description of *Marinilabilia salmonicolor* with *Marinilabilia agarovorans* as its subjective synonym. *Int J Syst Bacteriol* **49**, 1551–1557.
- Swofford, D. L. (1998). Phylogenetic analysis using parsimony (PAUP), version 4. Sunderland, MA: Sinauer Associates.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Veldkamp, H. (1961). A study of two marine agar-decomposing, facultatively anaerobic myxobacteria. *J Gen Microbiol* **26**, 331–342.
- Zhilina, T. N., Appel, R., Probian, C., Brossa, E. L., Harder, J., Widdel, F. & Zavarzin, G. A. (2004). *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. *Arch Microbiol* **182**, 244–253.