Description of *Persicirhabdus sediminis* gen. nov.,
sp. nov., *Roseibacillus ishigakijimensis* gen. nov.,
sp. nov., *Roseibacillus ponti* sp. nov., *Roseibacillus
persicicus* sp. nov., *Luteolibacter pohnpeiensis*
gen. nov., sp. nov. and *Luteolibacter algae* sp. nov.,
six marine members of the phylum
'*Verrucomicrobia*', and emended descriptions of
the class *Verrucomicrobiae*, the order
Verrucomicrobiales and the family
Verrucomicrobiaceae

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Ten pale-pink- and pale-yellow-pigmented, Gram-negative, non-motile, rod-shaped, chemoheterotrophic bacteria designated strains YM20-087^T, YM21-151, MN1-741^T, YM27-120^T, YM26-010^T, YM24-184, YM20-122, A4T-83^T, A5J-41-2^T and A5J-40 were isolated from various marine environments and were subjected to a polyphasic taxonomic investigation. Phylogenetic analyses based on 16S rRNA gene sequences indicated that these isolates belonged to the phylum '*Verrucomicrobia*' (subdivision 1) and represented three independent lineages that were distinct from species of genera of the family *Verrucomicrobiaceae* with validly published names. The cell-wall peptidoglycan of these strains contained muramic acid and meso-diaminopimelic acid. Strains MN1-741^T, YM27-120^T, YM26-010^T, YM24-184 and YM20-122 produced pinkish carotenoid pigments. On the basis of polyphasic taxonomic evidence, it was concluded that these strains should be classified within three new genera, *Persicirhabdus* gen. nov. (with one species, the type species *Persicirhabdus sediminis* sp. nov.), *Roseibacillus* gen. nov. (with three species; type species *Roseibacillus ishigakijimensis* sp. nov.) and *Luteolibacter* gen. nov. (with two species; type species *Luteolibacter pohnpeiensis* sp. nov.), of the family *Verrucomicrobiaceae* within the phylum '*Verrucomicrobia*'. The names *Persicirhabdus sediminis* gen. nov., sp. nov. (type strain YM20-087^T = MBIC08313^T = KCTC 22039^T), *Roseibacillus ishigakijimensis* gen. nov., sp. nov. (type strain MN1-741^T = MBIC08315^T = KCTC 12986^T), *Roseibacillus ponti* sp. nov. (type strain YM27-120^T = MBIC08316^T = KCTC 12987^T), *Roseibacillus persicicus* sp. nov. (type strain YM26-010^T = MBIC08317^T = KCTC 12988^T), *Luteolibacter pohnpeiensis* gen. nov., sp. nov. (type strain A4T-83^T = MBIC08322^T = KCTC 22041^T) and *Luteolibacter algae* sp. nov. (type strain A5J-41-2^T = MBIC08320^T = KCTC 22040^T) are therefore proposed. Emended descriptions of the class *Verrucomicrobiae*, the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae* are also presented.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YM20-087^T, MN1-741^T, YM27-120^T, YM26-010^T, A4T-83^T and A5J-41-2^T are AB331886, AB331888, AB331889, AB331890, AB331895 and AB331893, respectively.

Compositions of complex media used during isolation of strains and detailed Biolog GN2 test results are available as supplementary material with the online version of this paper.

The phylum 'Verrucomicrobia' (Hedlund *et al.*, 1997; Hugenholtz *et al.*, 1998) of the domain *Bacteria* has been divided into six monophyletic subdivisions (Hugenholtz *et al.*, 1998; Vandekerckhove *et al.*, 2000), of which subdivision 1 is listed as the family *Verrucomicrobiaceae* in the second edition of *Bergey's Manual of Systematic Bacteriology* (Garrrity & Holt, 2001). Members of subdivision 4 have recently been formally proposed as the class *Opitutae*, which is composed of two orders; the order *Puniceicoccales* containing the family *Puniceicoccaceae* and the order *Opitutales* containing the family *Opitutaceae* (Choo *et al.*, 2007). However, owing to the presence of relatively few pure cultured and characterized species, the classification of the phylum 'Verrucomicrobia' is still ambiguous and informal. For this reason, for formal classification of the phylum 'Verrucomicrobia', it is recommended that many verrucomicrobia that 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; Dedysh *et al.*, 2006; Haukka *et al.*, 2006) should be isolated and cultivated. Moreover, the remaining subdivisions must be proposed formally by the rank of class.

Strains investigated and source samples are listed in Table 1. Samples (0.5–1 cm³) were homogenized with a glass rod in 5 ml sterile seawater. The homogenate (50 µl) was applied to each medium (Table 1; also see Supplementary Tables S1 and S2 available in IJSEM Online) for isolation. Bacteria

were purified on marine broth 2216 (Difco) containing 1.5 % agar. Strain MN1-741^T was isolated by using an *in situ* cultivation technique (Yasumoto-Hirose *et al.*, 2006) from an artificial polyurethane foam (PUF) block supplemented with HV agar (Hayakawa & Nonomura, 1987) in 70 % seawater containing 0.25 g lignin l⁻¹. The PUF block was placed in a coral reef area in Yonehara, Ishigakijima (an island in Okinawa), Japan, for 3 days in June 2006 and then maintained for 1 month in circulating seawater in Kamaishi, Japan. Strain isolation was done in the same way as described by Yoon *et al.* (2007a) using 1/10-strength marine agar 2216 (MA) (Difco) containing 50 µg cycloheximide ml⁻¹ and 40 µl nalidixic acid ml⁻¹.

In the present study, we attempted to elucidate the phylogenetic position of strains YM20-087^T, YM21-151, MN1-741^T, YM27-120^T, YM26-010^T, YM24-184, YM20-122, A4T-83^T, A5J-41-2^T and A5J-40 using a polyphasic taxonomic approach including 16S rRNA gene sequence analysis. In parallel, we performed physiological, biochemical and chemotaxonomic analyses to characterize the ten novel isolates. Based on these data, it is proposed that the isolates represent three new genera and six novel species of the family *Verrucomicrobiaceae* within the phylum 'Verrucomicrobia'. Additionally, with the inclusion of these isolates, we propose emended descriptions of the class *Verrucomicrobiae*, the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae* for formal classification of 'subdivision 1'.

Table 1. Strains used in this study

Medium P was described by Yoon *et al.* (2007a). The compositions of media R and MH are detailed in Supplementary Tables S1 and S2.

Strain	Source	Sampling site	Medium for isolation	Date
MN1-741 ^T	Seawater	Coral reef area in Yonehara, Ishigakijima (an island in Okinawa), Japan	1/10-strength MA containing 50 µg cycloheximide ml ⁻¹ and 40 µl nalidixic acid ml ⁻¹	June 2006
A5J-41-2 ^T	Red alga, unidentified	Estuary of Taira River, Nagasaki, Japan (GPS location 32° 49' 31.7" N 129° 46' 53.5" E)	1/10-strength nutrient agar	June 2005
A5J-40	Red alga, unidentified	As above	1/10-strength nutrient agar	June 2005
A4T-83 ^T	Driftwood	Mangrove coast, Pohnpei Island (06° 58' 48" N 158° 10' 36" E)	1/10-strength MA containing 20 p.p.m. korormicin (Yoshikawa <i>et al.</i> , 1997)	November 2004
YM20-087 ^T	Sediment	Shore of Sodegaura-Kaihinkouen, Sodegaura, Chiba, Japan (35° 27' 17.0" N 139° 57' 03.8" E)	Medium P	August 2005
YM20-122	Brown alga, <i>Laminaria</i> sp.	As above	Medium R	August 2005
YM21-151	Sediment	Shore of Natsudomari Peninsula, Aomori, Japan (41° 00' 14.5" N 140° 52' 50.5" E)	Medium P	September 2005
YM24-184	White sponge, unidentified	Uranouchi Bay, Suzaki, Kouchi, Japan (33° 26' 04.2" N 133° 24' 40.4" E)	Medium MH	May 2006
YM26-010 ^T	Iron sand	Touni Bay, Touni-cho, Kamaishi, Iwate, Japan (39° 11' 59.25" N 141° 52' 01.79" N)	Medium MH	July 2006
YM27-120 ^T	Brown alga, <i>Padina</i> sp.	Shore of Futatsugame, Washizaki, Sado, Niigata, Japan (38° 19' 43.6" N 138° 29' 09.6" E)	Medium P	August 2006

The temperature and pH ranges for growth were determined by incubating the isolates on 1/2-strength R2A agar (Difco) with 75 % artificial seawater (Lyman & Fleming, 1940), MA, 1/5-strength MA and 1/10-strength MA. The NaCl concentration for growth was determined on 1/2-strength R2A agar 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) and transmission electron microscopy (TEM). For TEM observation, cells were mounted on Formvar-coated copper grids and negatively stained with 1 % (w/v) aqueous uranyl acetate. Grids were observed in a JEOL 1011 TEM operated at 100 kV. In the course of TEM, various cell sizes were observed. Cells of strains YM20-087^T and YM21-151 grown on MA were rod-shaped and generally 0.5–0.8 μm wide and 1.0–1.5 μm long. Cells of strains MN1-741^T, YM27-120^T, YM26-010^T, YM24-184 and YM20-122 on 1/5-strength MA were rod-shaped and generally 0.7–0.8 μm wide and 1.0–1.5 μm long (Fig. 1). Finally, cells of strains A4T-83^T, A5J-41-2^T and A5J-40 on 1/10-strength MA were rod-shaped, 0.5–0.8 μm wide and 1.0–2.0 μm long. Cells of the ten strains divided by means of binary fission, but cells were non-motile and flagella and appendages were not observed. Growth under anaerobic conditions was determined after 2 weeks of incubation in an AnaeroPack (Mitsubishi Gas Chemical Co.) on 1/2-strength R2A agar with 75 % artificial seawater, MA, 1/5-strength MA and 1/10-strength 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 physiological and biochemical characteristics. All suspension media for API test strips were supplemented with 0.85 % (w/v) NaCl solution (final concentration). API 20NE and API ZYM were read after 48 h incubation at 30 °C and 4 h incubation at 37 °C, respectively. The substrate

Fig. 1. Transmission electron micrograph of negatively stained cells of strain MN1-741^T. Bar, 1 μm .

oxidation ranges of the strains were determined using Biolog MicroPlates (Supplementary Table S3). Determination of respiratory quinone systems and cellular fatty acid compositions was carried out as described previously (Katsuta *et al.*, 2005). DNA was prepared according to the method of Marmur (1961) 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 50 °C. Hybridization was performed using five replications; of the values obtained, the highest and lowest for each sample was excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values. Cell walls were prepared by the methods described by Schleifer & Kandler (1972) and amino acids in an acid hydrolysate of the cell walls were identified by TLC (Harper & Davis, 1979) and by 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 27F and 1492R (*Escherichia coli* numbering system; Weisburg *et al.*, 1991) specific to the 16S rRNA gene. To ascertain the phylogenetic position of the new isolates, the 16S rRNA gene sequences of the investigated strains 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, and 1123 bases of the 16S rRNA gene sequence were compared. Aligned sequences were analysed by using the MEGA 3.1 software (Kumar *et al.*, 2004). Evolutionary distances [distance options according to Kimura's 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). 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 three different monophyletic clades within the family *Verrucomicrobiaceae* (Fig. 2). The phylogenetic tree showed that the ten novel isolates were clustered within the family *Verrucomicrobiaceae* of the phylum '*Verrucomicrobia*' with bootstrap confidence values of more than 90 % in the neighbour-joining method (Fig. 2) and more than 80 % in the maximum-parsimony method (data not shown). Strains YM20-087^T and YM21-151 had sequences that were 100 % identical and were related to the genus *Rubritalea* (sequence similarities 90.5–91.6 %). Strain MN1-741^T, YM27-120^T, YM26-010^T, YM24-184 and YM20-122 formed a single cluster with sequence similarities between the strains ranging

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of the novel taxa described in this study among the currently known and cultivated species of the family Verrucomicrobiaceae within the phylum 'Verrucomicrobia'. Numbers at nodes indicate bootstrap values derived from 1000 replications. Numbers in parentheses are GenBank/EMBL/DBJ accession numbers. Sequences determined in this study are shown in bold. The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup reference. Bar, 2 % sequence divergence.

from 98.7 to 99.9 % (Fig. 2). Strains YM26-010^T, YM24-184 and YM20-122 were 99.9 % similar to each other, while the other strains were slightly more distant. The 16S rRNA gene sequence similarity between strains A5J-41-2^T and A5J-40 was 100 %, and these strains possessed 96.1 % sequence similarity to strain A4T-83^T. All other novel isolates and so far cultivated species of the family Verrucomicrobiaceae with validly published names were more distantly related, showing sequence similarity of less than 90 %.

DNA–DNA hybridization values between strain MN1-741^T and strains YM27-120^T and YM26-010^T were 2.9 and 1.3 %, respectively. These results strongly suggest that strains MN1-741^T, YM27-120^T and YM26-010^T should be classified as three separate species (Wayne *et al.*, 1987). DNA–DNA relatedness values of 89–96 % between strains YM26-010^T, YM24-184 and YM20-122 indicate that these strains represent members of the same species.

A pinkish pigment accumulated in the cells was extracted by acetone after freeze-drying treatment of the cells, and analysed by HPLC/PAD (photodiode array detection). This

pigment showed UV-visible spectra with absorption maxima at 494 and 524 nm (strain MN1-741^T), 490 and 530 nm (strain YM27-120^T), 494 and 523 nm (strain YM26-010^T), 494 and 521 nm (strain YM24-184) and 494 and 523 nm (strain YM20-122), characteristic of carotenoids. As shown in Table 2, the predominant fatty acids separated the novel strains into three separate groups. The major fatty acids of strains YM20-087^T and YM21-151 were C_{14:0} (9.7–11.7 %), C_{16:0} (54.7–59.6 %) and C_{16:1ω7c} (22.1–23.2 %). The major fatty acids of strains MN1-741^T, YM27-120^T, YM26-010^T, YM24-184 and YM20-122 were C_{16:0} (18.6–38.6 %), C_{16:1ω9c} (16.5–25.2 %) and iso-C_{14:0} (16.5–37.5 %). For strains A4T-83^T, A5J-41-2^T and A5J-40, the major fatty acids were C_{16:0} (15.6–20.2 %) and iso-C_{14:0} (43.0–45.0 %). The fatty acid profiles of the strains studied discriminated them from other members of the family Verrucomicrobiaceae. In strain YM27-120^T, a fatty acid unidentified in the MIDI database was detected at a composition of 11 % by GC/MS analysis. This fatty acid was assumed to be C_{16:1} or cyclo C_{16:0} on the basis of the MS spectra.

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

Data for *Rubritalea* species were taken from Scheuermayer *et al.* (2006), Kasai *et al.* (2007) and Yoon *et al.* (2007b). Data are expressed as percentages of total fatty acids. Fatty acids representing less than 1% are not shown. —, Not detected; tr, trace.

Fatty acid	YM20-087 ^T	YM21-151	MN1-741 ^T	YM27-120 ^T	YM26-010 ^T	YM24-184	YM20-122	A4T-83 ^T	A5J-41-2 ^T	A5J-40	<i>Rubritalea</i> species
Saturated											
C _{13:0}	—	—	—	—	—	—	—	—	tr	tr	—
C _{14:0}	11.7	9.7	tr	1.3	5.5	tr	2	4.4	13.9	12.5	tr–2.9
C _{15:0}	—	1.1	1.4	4	5	2.5	1.4	1.9	2.4	3.4	1.4–4.5
C _{16:0}	59.6	54.7	20.8	38.6	27.9	18.6	35.5	15.6	17.1	20.2	4.4–23.1
C _{17:0}	—	—	2.2	3.1	5.5	6.3	1.9	tr	tr	tr	tr–2.8
C _{18:0}	—	1.9	2.4	1.3	1.3	tr	tr	—	1.3	1.2	tr–2.4
Unsaturated											
C _{14:1ω5c}	—	—	—	—	—	—	—	—	3	1.6	—
C _{14:1ω7c}	5.5	4.2	—	—	—	—	—	—	—	—	—
C _{15:1ω6c}	—	—	—	—	—	—	—	—	—	—	tr–2
C _{16:1ω7c}	23.2	22.1	—	1.4	1.1	—	—	6.7	8.2	9.9	7–21.5
C _{16:1ω9c}	—	—	20	12.2	25.2	20.8	16.5	—	—	—	—
C _{17:1ω8c}	—	—	tr	tr	1.2	1	—	—	—	—	—
C _{18:1ω7c}	—	—	—	—	—	—	—	—	—	—	1.5
C _{18:1ω9c}	—	—	1.3	1.8	1.4	tr	—	—	—	—	—
Branched											
iso-C _{14:0}	—	2.7	37.5	16.5	20.5	35.4	30.9	45	45.4	43	22–43.1
iso-C _{16:0}	—	tr	9.1	3.9	2	9.7	8.6	14.8	2.3	2.4	12.2–20.6
iso-C _{18:0}	—	—	3.2	tr	—	1.6	tr	—	—	—	—
anteiso-C _{15:0}	—	1.6	1.1	1.8	2.3	tr	2.2	10.5	4.7	3.7	4.7–18.1
anteiso-C _{17:0}	—	—	—	—	—	—	—	tr	—	—	1.6–2
Unidentified											
C _{16:1/cyclo}	—	—	—	11	—	—	—	—	—	—	—
C _{16:0}	—	—	—	—	—	—	—	—	—	—	—

The novel isolates could be distinguished from the other taxa of the class *Verrucomicrobiae* by the phenotypic characteristics given in Table 3. Cell walls of the ten novel isolates were prepared by disrupting cells followed by heating with 3% SDS, washing and centrifugation. Amino acid analysis of the cell-wall hydrolysates indicated the presence of muramic acid and *meso*-diaminopimelic acid in the cell-wall peptidoglycan of these two isolates.

Based on the results of our phylogenetic analysis and their biochemical and physiological properties, the ten novel strains studied here (Table 1) represent three new genera and six novel species of the family *Verrucomicrobiaceae*. The names *Persicirhabdus sediminis* gen. nov., sp. nov. (type strain YM20-087^T), *Roseibacillus ishigakijimensis* gen. nov., sp. nov. (type strain MN1-741^T), *Roseibacillus ponti* sp. nov. (type strain YM27-120^T), *Roseibacillus persicicus* sp. nov. (type strain YM26-010^T), *Luteolibacter pohnpeiensis* gen. nov., sp. nov. (type strain A4T-83^T) and *Luteolibacter algae* sp. nov. (type strain A5J-41-2^T) are proposed.

Emended description of the class *Verrucomicrobiae* Hedlund *et al.* 1998

Equivalent to 'subdivision 1' (Hugenholtz *et al.*, 1998) of the phylum '*Verrucomicrobia*' and is defined by phylogenetic

analyses based on 16S rRNA gene sequences obtained from several cultivated members and a wide range of uncultivated bacteria retrieved mainly from aquatic and marine habitats. Gram-negative. The class comprises the order *Verrucomicrobiales* and the family *Verrucomicrobiaceae*. The type order is the order *Verrucomicrobiales* Ward-Rainey *et al.* 1996.

Emended description of the order *Verrucomicrobiales* Ward-Rainey *et al.* 1996

Encompasses Gram-negative bacteria retrieved mainly from freshwater and marine environments, within the class *Verrucomicrobiae*. The order contains the family *Verrucomicrobiaceae*. Delineation of the order is determined primarily by phylogenetic information from 16S rRNA gene sequences. The nomenclatural type is the genus *Verrucomicrobium* Schlesner 1988.

Emended description of the family *Verrucomicrobiaceae* Ward-Rainey *et al.* 1996

Encompasses Gram-negative bacteria retrieved mainly from terrestrial and freshwater sources (ponds, lakes and human faeces) and marine sources (seawater, sediment, marine fauna and flora), within the order *Verrucomicrobiales*.

Table 3. Phenotypic characteristics of strains belonging to the new genera *Persicirhabdus*, *Roseibacillus* and *Luteolibacter* compared with related taxa within the class *Verrucomicrobiae*

Reference strains: 1, *Prostheobacter fusiformis* FC4^T (data from Staley *et al.*, 1976; Hedlund *et al.*, 1996); 2, *Verrucomicrobium spinosum* DSM 4136^T (Schlesner, 1987); 3, *Akkermansia muciniphila* Muc^T (Derrien *et al.*, 2004); 4, *Rubritalea marina* Pol012^T (Scheuermayer *et al.*, 2006). +, Positive; w, weakly positive; −, negative; ND, no data.

Characteristic	YM20-087 ^T	YM21-151	MN1-741 ^T	YM27-120 ^T	YM26-010 ^T	YM24-184	YM20-122	A4T-83 ^T	A5J-41-2 ^T	A5J-40	1	2	3	4
Isolation source	Sediment	Sediment	Seawater	Brown alga	Iron sand	White sponge	Brown alga	Driftwood	Red algae	Red algae	Marl pond	Lake	Human faeces	Sponge
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Fusiform rod	Fusiform rod	Oval-shaped	Coccoid or rod
Colony colour	Pale pink	Pale pink	Pale pink	Reddish pink	Pink	Reddish pink	Reddish pink	Pale yellow	Pale yellow	Pale yellow	Opaque yellow	Light yellow	White	Red
Tolerance of oxygen	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Prosthecate	−	−	−	−	−	−	−	−	−	−	+	+	−	−
Fimbriae	−	−	−	−	−	−	−	−	−	−	+	+	−	−
Production of:														
Catalase	−	−	+	w	w	w	w	+	+	+	+	+	ND	−
Oxidase	+	+	+	+	+	+	+	+	+	+	−	+	ND	+
Temperature range (°C)	20–30	20–30	20–37	15–37	15–37	15–37	15–37	20–37	4–37	4–37	4–40	26–34	20–40	8–30
Growth at 4 °C	−	−	−	−	−	−	−	−	+	+	+	−	−	−
Growth at 37 °C	−	−	+	+	+	+	+	+	+	+	+	−	+	−
pH range	6.5–8.5	6.5–9	6.5–9	7–9	6.5–9	6.5–9	6.5–9	6.5–9	6.5–8.5	6.5–9	ND	9	5.5–8	6.8–8.2
NaCl tolerance (%)	2.5	2.5	6	7	7	7	5	6	3	4	ND	1	ND	3.8
NaCl requirement	−	−	+	+	+	+	+	+	−	−	ND	−	ND	+
DNA G + C content (mol%)	50.4	50.4	61.4	57.6	58.3	57.3	57.4	55.8	55.9	55.5	56.1	58.6	47.6	50.9
Major quinone(s)	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9, MK-10	MK-9	MK-9	ND	ND	ND	MK-8, MK-9

Currently, the family includes the genera *Prostheco bacter*, *Verrucomicrobium*, *Akkermansia* and *Rubritalea* and the three new genera described in this study, *Persicirhabdus*, *Roseibacillus* and *Luteolibacter*, together with several uncultivated aquatic and marine bacteria. Delineation of the order is determined primarily by phylogenetic information from 16S rRNA gene sequences. The nomenclatural type is the genus *Verrucomicrobium* Schlesner 1988.

Description of *Persicirhabdus* gen. nov.

Persicirhabdus (Per.si.ci.rhab'dus. L. n. *persicum* a peach; N.L. fem. n. *rhabdus* from Gr. fem. n. *rhabdos* a rod; N.L. fem. n. *Persicirhabdus* peach-coloured rod, because the colonies are peach-coloured).

Cells are rod-shaped, Gram-negative and obligately aerobic. Cells lack flagella and are non-motile. No spores are formed. Catalase reaction is negative, whereas oxidase is positive. Nitrate is reduced to nitrite. The major respiratory quinone is MK-9. The G+C content of the genomic DNA of the type strain of the type species is 50.4 mol%. Predominant cellular fatty acids are C_{14:0}, C_{16:0} and C_{16:1ω7c}. The type species is *Persicirhabdus sediminis*.

Description of *Persicirhabdus sediminis* sp. nov.

Persicirhabdus sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment).

Main characteristics are the same as those given for the genus. In addition, cells are 0.5–0.8 μm wide and 1.0–1.5 μm long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on MA are circular, convex and pale pink in colour. The temperature range for growth is 20–30 °C, with optimal growth at 25–30 °C. No growth occurs at either 4 or 45 °C. The pH range for growth is 6.5–8.5. NaCl is not required for growth, but is tolerated up to 2.5 % (w/v). Alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase tests are positive, but esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase tests are negative. Dextrin, Tween 80, L-arabinose, trehalose and glucose 6-phosphate are oxidized. The cell-wall peptidoglycan contains muramic acid and meso-diaminopimelic acid. Major fatty acid components (>1.0 %) include C_{14:0} (11.7 %), C_{16:0} (59.6 %), C_{14:1ω7c} (5.5 %) and C_{16:1ω7c} (23.2 %).

The type strain is YM20-087^T (=MBIC08313^T =KCTC 22039^T), which was isolated from marine sediment.

Description of *Roseibacillus* gen. nov.

Roseibacillus (Ro.se.i.ba.cil'lus. L. adj. *roseus* rose-coloured, rosy; L. masc. n. *bacillus* a small staff or rod; N.L. masc. n. *Roseibacillus* a rose-coloured rod).

Cells are rod-shaped, Gram-negative and obligately aerobic. Cells lack flagella and are non-motile. No spores are formed. Catalase- and oxidase-positive. Nitrate is not reduced. The major respiratory menaquinone is MK-9. The G+C contents of the genomic DNA of known strains are 57.3–61.4 mol%. Predominant cellular fatty acids are C_{16:0}, C_{16:1ω9c} and iso-C_{14:0}. The type species is *Roseibacillus ishigakijimensis*.

Description of *Roseibacillus ishigakijimensis* sp. nov.

Roseibacillus ishigakijimensis (i.shi.ga.ki.ji.men'sis. N.L. masc. adj. *ishigakijimensis* pertaining to Ishigakijima, an island in Okinawa, from where the type strain was isolated).

Main characteristics are the same as those given for the genus. In addition, cells are 0.7–0.8 μm wide and 1.0–1.5 μm long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA are circular, convex and pale pink in colour. The temperature range for growth is 20–37 °C, with optimal growth at 30–37 °C. No growth occurs at either 4 or 45 °C. The pH range for growth is 6.5–9.0. NaCl is required for growth and can be tolerated up to 6 % (w/v). Alkaline phosphatase, esterase (C4) and naphthol-AS-BI-phosphohydrolase tests are positive, but leucine arylamidase, acid phosphatase, N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, valine arylamidase, trypsin, esterase lipase (C8), lipase (C4), cystine arylamidase, chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase tests are negative. Tween 80, L-arabinose, D-glucose, lactulose, maltose and DL-lactic acid are oxidized. The cell-wall peptidoglycan contains muramic acid and meso-diaminopimelic acid. Major fatty acid components (>1.0 %) include iso-C_{14:0} (37.5 %), anteiso-C_{15:0} (1.1 %), C_{15:0} (1.4 %), iso-C_{16:0} (9.1 %), C_{16:1ω9c} (20.0 %), C_{16:0} (20.8 %), C_{17:0} (2.2 %), iso-C_{18:0} (3.2 %), C_{18:1ω9c} (1.3 %) and C_{18:0} (2.4 %). The G+C content of the genomic DNA of the type strain is 61.4 mol%.

The type strain is MN1-741^T (=MBIC08315^T =KCTC 12986^T), which was isolated from seawater by using an *in situ* cultivation technique.

Description of *Roseibacillus ponti* sp. nov.

Roseibacillus ponti (pon'ti. L. gen. n. *ponti* of the sea).

Main characteristics are the same as those given for the genus. In addition, cells are 0.7–0.8 μm wide and 1.2–1.5 μm long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/2-strength R2A agar with 75 % artificial seawater are circular, convex and reddish pink in colour. The temperature range for growth is 15–37 °C, with optimal growth at 30–37 °C. No growth occurs at either 4 or 45 °C. The pH range for

growth is 7.0–9.0. NaCl is required for growth and can be tolerated up to 7% (w/v). Alkaline phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase tests are positive, but esterase (C4), leucine arylamidase, acid phosphatase, *N*-acetyl- β -glucosaminidase, α -galactosidase, β -galactosidase, α -glucosidase, valine arylamidase, trypsin, lipase (C4), cystine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase tests are negative. Dextrin, L-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, lactulose, D-mannitol, D-mannose, furanose, D-galacturonic acid, α -ketoglutaric acid, DL-carnitine, γ -aminobutyric acid, uridine, thymidine, 2,3-butanediol, glycerol and DL- α -glycerol phosphate are oxidized. The cell-wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include iso-C_{14:0} (16.5%), C_{14:0} (1.3%), anteiso-C_{15:0} (1.8%), C_{15:0} (4.0%), iso-C_{16:0} (3.9%), C_{16:0} (38.6%), C_{16:1 ω 7c} (1.4%), C_{16:1 ω 9c} (12.2%), C_{16:1} (11.0%), C_{17:0} (3.1%), C_{18:0} (1.3%) and C_{18:1 ω 9c} (1.8%). The G+C content of the genomic DNA of the type strain is 57.6 mol%.

The type strain is YM27-120^T (=MBIC08316^T =KCTC 12987^T), which was isolated from a brown alga.

Description of *Roseibacillus persicus* sp. nov.

Roseibacillus persicus (per.si'ci.cus. L. n. *persicum* a peach; L. suff. *-icus -a -um* suffix used with the sense of belonging to; N.L. masc. adj. *persicus* belonging to peach, because the bacterium is peach-coloured).

Main characteristics are the same as those given for the genus. In addition, cells are 0.7–0.8 μ m wide and 1.0–1.5 μ m long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5-strength MA are circular, convex and pink in colour. The temperature range for growth is 15–37 °C, with optimal growth at 30–37 °C. No growth occurs at either 4 or 45 °C. The pH range for growth is 6.5–9.0. NaCl is required for growth and can be tolerated up to 7% (w/v). Alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase tests are positive, but 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 tests are negative. Dextrin, glycogen, L-arabinose, cellobiose, D-fructose, D-galactose, D-mannose, trehalose, furanose, xylitol, succinic acid, L-glutamic acid, DL-carnitine, urocanic acid, DL- α -glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate are oxidized. The cell-wall peptidoglycan contained muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include iso-C_{14:0} (20.5%), C_{14:0} (5.5%), anteiso-C_{15:0} (2.3%), C_{15:0} (5.0%), iso-C_{16:0} (2.0%), C_{16:1 ω 9c} (25.2%), C_{16:1 ω 7c} (1.1%), C_{16:0} (27.9%), C_{17:1 ω 8c} (1.2%), C_{17:0} (5.5%), C_{18:1 ω 9c} (1.4%) and C_{18:0} (1.3%). The G+C content of the genomic DNA of the type strain is 58.3 mol%.

The type strain is YM26-010^T (=MBIC08317^T =KCTC 12988^T), which was isolated from iron sand.

Description of *Luteolibacter* gen. nov.

Luteolibacter (Lu.te.o.li.bac'ter. L. adj. *luteolus* pale yellow; N.L. masc. n. *bacter* from Gr. neut. n. *baktron* rod; N.L. masc. n. *Luteolibacter* a pale-yellow-coloured rod).

Cells are rod-shaped, Gram-negative. Cells lack flagella and are non-motile. No spores are formed. Catalase- and oxidase-positive. Nitrate is not reduced. The major respiratory quinone is MK-9. The G+C contents of the genomic DNA of known strains are around 55 mol%. Predominant cellular fatty acids are C_{16:0} and iso-C_{14:0}. The type species is *Luteolibacter pohnpeiensis*.

Description of *Luteolibacter pohnpeiensis* sp. nov.

Luteolibacter pohnpeiensis (poh.n.pe.i.en'sis. N.L. masc. adj. *pohnpeiensis* pertaining to Pohnpei Island, located in Micronesia, where the type strain was isolated).

Main characteristics are the same as those given for the genus. In addition, cells are 0.5–0.8 μ m wide and 1.5–2.0 μ m long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/10-strength MA are circular, convex and pale yellow in colour. The temperature range for growth is 20–37 °C, with optimal growth at 30–37 °C. No growth occurs at either 4 or 45 °C. The pH range for growth is 6.5–9.0. NaCl is required for growth and can be tolerated up to 6% (w/v). Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase and α -mannosidase tests are positive, but α -glucosidase, esterase (C4), lipase (C4), cystine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase and α -fucosidase tests are negative. Dextrin, L-arabinose, cellobiose, i-erythritol, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, lactulose, maltose, D-mannose, melibiose, methyl β -D-glucoside, D-psicose, raffinose, D-sorbitol, sucrose, trehalose, furanose, glycerol and glucose 6-phosphate are oxidized. MK-10 is also present as a major respiratory menaquinone. The cell-wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{14:0} (4.4%), C_{15:0} (1.5%), C_{16:0} (15.6%), C_{16:1 ω 7c} (6.7%), iso-C_{14:0} (45.0%), iso-C_{16:0} (14.8%) and anteiso-C_{15:0} (10.5%). The G+C content of the genomic DNA of the type strain is 55.8 mol%.

The type strain is A4T-83^T (=MBIC08322^T =KCTC 22041^T), which was isolated from a piece of driftwood from a mangrove coast located on Pohnpei Island.

Description of *Luteolibacter algae* sp. nov.

Luteolibacter algae (al'gae. L. fem. gen. n. *algae* of an alga, referring to the source of isolation of the type strain).

Main characteristics are the same as those given for the genus. In addition, cells are 0.5–0.7 µm wide and 1.0–1.5 µm long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/10-strength MA are circular, convex and pale yellow in colour. The temperature range for growth is 4–37 °C, with optimal growth at 20–30 °C. No growth occurs at 45 °C. The pH range for growth is 6.5–8.5. NaCl is not required for growth, but is tolerated up to 3% (w/v). Alkaline phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase tests are positive, but leucine arylamidase, trypsin, acid phosphatase, α -glucosidase, 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 tests are negative. Dextrin, Tween 80, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, lactulose, D-mannose, melibiose, D-psicose, monomethyl succinate, formic acid, D-galacturonic acid and 2,3-butanediol are oxidized. The cell-wall peptidoglycan contains muramic acid and *meso*-diaminopimelic acid. Major fatty acid components (>1.0%) include C_{14:0} (13.9%), C_{15:0} (2.4%), C_{16:0} (17.1%), C_{18:0} (1.3%), C_{14:1 ω 5c} (3.0%), C_{16:1 ω 7c} (9.9%), iso-C_{14:0} (45.4%), iso-C_{16:0} (2.3%) and ante-iso-C_{15:0} (4.7%). The G+C content of the genomic DNA of the type strain is 55.9 mol%.

The type strain is A5J-41-2^T (=MBIC08320^T =KCTC 22040^T), which was isolated from an unidentified red alga.

Acknowledgements

The authors would like to express their gratitude to Atsuko Katsuta, Ayako Matsuzaki, Tomomi Haga and Yukiko Itazawa for their technical assistance. This work was supported by the New Energy and Industrial Technology Development Organization (NEDO).

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