Pontibacter actiniarum gen. nov., sp. nov., a novel member of the phylum 'Bacteroidetes', and proposal of *Reichenbachiella* gen. nov. as a replacement for the illegitimate prokaryotic generic name *Reichenbachia* Nedashkovskaya *et al.* 2003

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The taxonomic position of a marine, gliding, pink-pigmented, aerobic, heterotrophic and Gram-negative bacterium was established using a polyphasic approach. 16S rRNA gene sequence analysis indicated that the strain was a member of the phylum 'Bacteroidetes' in which it occupied a separate lineage. The predominant cellular fatty acids were C15:0 iso, C17:0 iso 3-OH, summed feature 3 and summed feature 4. The DNA G+C content was 48.7 mol%. Phylogenetic evidence and the results of phenotypic, genotypic and chemotaxonomic analyses strongly support the assignment of the newly isolated bacterium as a member of a novel genus and species, for which the name *Pontibacter actiniarum* gen. nov., sp. nov. is proposed. The type strain is KMM 6156^T (=KCTC 12367^T=LMG 23027^T). It is also proposed that the illegitimate names *Reichenbachia* and *Reichenbachia agariperforans* are replaced with *Reichenbachiella* and *Reichenbachiella agariperforans*, respectively.

Marine bacteria of the phylum 'Bacteroidetes' with menaquinone 7 (MK-7) as their main respiratory quinone are frequently found in marine ecosystems. They can move by means of gliding or be non-motile and are rod-shaped or ring-like. The majority of them have been isolated from sea water, sediment or algae (Bowman *et al.*, 2003; Brettar *et al.*,

2004a, b; Nedashkovskaya *et al.*, 2003, 2004, 2005a; Raj & Maloy, 1990; Van Trappen *et al.*, 2004; Yi & Chun, 2004; Yoon *et al.*, 2004, 2005a, b, c). Currently, only two species with validly published names that belong to the MK-7 phylogenetic cluster, *Cyclobacterium marinum* (formerly *Flectobacillus marinus*) and *Roseivirga echinomitans*, have been isolated from marine animals: sea urchins of *Dendraster* sp. and *Strongylocentrotus intermedius*, respectively (Larkin & Borrall, 1984; Nedashkovskaya *et al.*, 2005b).

In this study, we report the isolation and identification of a strain of heterotrophic, Gram-negative, aerobic, gliding and pink-coloured bacteria associated with a marine coelenterate. Based on a polyphasic study of strain KMM 6156^T,

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Abbreviation: MK-7, menaquinone 7.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Pontibacter actiniarum* sp. nov. KMM 6156^T is AY989908.

A scanning electron micrograph of cells of strain KMM 6156^T is available as a supplementary figure in IJSEM Online.

including phylogenetic, genotypic, chemotaxonomic and phenotypic analyses, a novel genus, *Pontibacter*, is proposed.

Strain KMM 6156^T was isolated from unidentified actinians, collected from a depth of 118 m in Rudnaya Bay, East Sea (also known as the Sea of Japan), Pacific Ocean, during July 2003. For strain isolation, 0.1 ml homogenate of actinian tissues was transferred onto plates of marine agar 2216 (Difco). After primary isolation and purification, strains were cultivated at 28 °C on the same medium and stored at –80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

Phylogenetic analysis

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed previous procedures (Kim *et al.*, 1998). Sequence data obtained were aligned with those of representative members of the phylum 'Bacteroidetes' by using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using suitable programs from the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated using the Kimura two-parameter model (Kimura, 1980) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain KMM 6156^T (1435 nucleotides) revealed that the strain belongs to the phylum 'Bacteroidetes', in which it forms a distinct lineage (Fig. 1). The type strain of the single species of the genus *Adhaeribacter*, *Adhaeribacter aquaticus* MBRG 1.5^T, was the closest relative of strain KMM 6156^T, with a 16S rRNA gene sequence similarity of 89.2 %.

Genotypic methods

DNA was isolated following the method of Marmur (1961) and the DNA G + C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C composition of KMM 6156^T was 48.7 mol%.

Chemotaxonomic methods

For the determination of the cellular fatty acid methyl ester content, strain KMM 6156^T was grown on marine agar at 25 °C for 48 h. FAME analysis was carried out according to the standard Microbial Identification System protocol (Microbial ID).

Strain KMM 6156^T is characterized by the presence of the predominant branched-chain saturated and unsaturated fatty acids C15:1 iso (28.8 %), C17:0 iso 3-OH (6.5 %), summed feature 3 (14.7 %; comprising C15:0 iso 2-OH

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain KMM 6156^T and representative members of the phylum 'Bacteroidetes'. Asterisks indicate branches that were also recovered using the maximum-likelihood algorithm. Numbers at nodes indicate bootstrap values (%). Bar, 0.01 substitutions per nucleotide position.

and/or C16:1 ω7) and summed feature 4 (31.3 %; consisting of C17:1 iso I and/or C17:1 anteiso B) (Table 1).

Isoprenoid quinones were extracted from lyophilized cells and analysed as described by Akagawa-Matsushita *et al.* (1992). Menaquinones were detected by monitoring at 270 nm and were identified by comparison with known quinones from the reference strain *Cyclobacterium marinum* LMG 13164^T. The main isoprenoid quinone was MK-7.

Phenotypic methods

The physiological and biochemical properties of strain KMM 6156^T were examined as described by Nedashkovskaya *et al.* (2003, 2004). Physiological and biochemical features of KMM 6156^T were determined using the API 20E, API 20NE, API ZYM and API 50 CH galleries (bioMérieux) and Biolog GN2 Microplate system (Biolog) according to the manufacturers' instructions. Susceptibility to antibiotics was tested as described previously (Nedashkovskaya *et al.*, 2003) and additional discs containing chloramphenicol

Table 1. Cellular fatty acid content of *Pontibacter actiniarum* KMM 6156^T and its closest relatives from the phylum 'Bacteroidetes'

Taxa: 1, *Pontibacter actiniarum* KMM 6156^T; 2, *Adhaeribacter aquaticus*; 3, *Hymenobacter actinosclerus*; 4, *Hymenobacter aerophilus*. Data from Buczolits *et al.* (2002), Nedashkovskaya *et al.* (2004), Rickard *et al.* (2005) and this study. Values are percentages and values of less than 1% are not shown. Summed feature 2 consisted of one or more of the following fatty acids which could not be separated by the Microbial Identification System: C15:1 iso I and C13:0 3-OH. Summed feature 3 consisted of one or more of the following fatty acids: C15:0 iso 2-OH, C16:1 ω 7c and C16:1 ω 7t. Summed feature 4 consisted of one or more of the following fatty acids: C17:1 iso I and C17:1 anteiso B. Summed feature 5 consisted of one or more of the following fatty acids: C18:0 anteiso and C18:2 ω 6,9c.

Fatty acid	1	2	3	4
C10:0 iso	—	1.2	—	—
C15:0 iso	28.8	22.5	22.3	10.8–15.0
C15:0 anteiso	0.1	4.4	25.8	18.6–22.3
C15:1 ω 6c	0.3	—	0.8	0.8–1.2
C16:1 ω 5c	0.8	16.9	3.7	6.6–7.9
C16:1 isoH	0.5	—	1.5	1.4–1.5
C16:0	0.2	—	—	1.6–1.7
C15:0 iso 2-OH	—	16.5	0.8	—
C15:0 iso 3-OH	3.0	3.1	1.6	1.3–1.6
C17:1 ω 6c	1.4	5.1	0.7	0–0.7
C17:0 iso	2.2	—	1.8	2.7–4.5
C17:0 anteiso	—	—	0.7	1.3–2.3
C17:0 iso 3-OH	6.5	12.1	3.1	2.6–3.5
C17:0 iso 2-OH	—	—	2.0	—
C17:0 2-OH	—	—	—	0.8–1.3
Summed feature 2	2.3	—	2.3	0.8–1.4
Summed feature 3	14.7	—	13.1	21.4–22.3
Summed feature 4	31.3	11.2	19.9	17.7–18.8
Summed feature 5	1.8	—	—	—

(30 μ g), doxycycline (10 μ g) and erythromycin (15 μ g) were used. Gliding motility was determined as described by Bowman (2000). To study cell morphology, samples were fixed in 2.5% paraformaldehyde–glutaraldehyde mixture buffered with 0.1 M phosphate (pH 7.2) for 2 h, fixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in graded ethanol and substituted by isoamyl acetate. Samples were then dried at the critical point in CO₂. Finally, the samples were sputtered with gold in a sputter coater (SC502; Polaron) and observed using a scanning electron microscope (SEM 515; Philips). Cells of KMM 6156^T ranged from 0.3 to 0.4 μ m in width and from 1.2 to 1.9 μ m in length (see Supplementary Fig. S1 in IJSEM Online).

Strain KMM 6156^T was Gram-negative, chemo-organotrophic, aerobic, pink-pigmented and motile by gliding. The main physiological and biochemical characteristics of the strain are given in Table 2 and the species description. The features that differentiate strain KMM 6156^T from related members of

the phylum 'Bacteroidetes' are shown in Table 2. Strain KMM 6156^T differs from its closest phylogenetic neighbour, the freshwater bacterium *Adhaeribacter aquaticus*, by its abilities to move by gliding and to hydrolyse agar, by the absence of amylase activity and by a higher DNA G+C content (48.7 mol% for KMM 6156^T compared with 40.0 mol% for *A. aquaticus*).

Strain KMM 6156^T could be distinguished from its closest relatives on the basis of phenotypic features, fatty acid content and significant phylogenetic distinctiveness. Low 16S rRNA gene sequence similarities of strain KMM 6156^T with the other members of the phylum 'Bacteroidetes' described to date (78.5–89.2%) clearly demonstrate that the newly isolated bacterium represents a novel genus.

Thus, the polyphasic data presented in this paper support the conclusion that strain KMM 6156^T can not be affiliated to any currently described taxa of the phylum 'Bacteroidetes'. Consequently, we propose that strain KMM 6156^T should be placed in a novel genus as *Pontibacter actiniarum* gen. nov., sp. nov.

Previously, we described the genus *Reichenbachia* and the novel species *Reichenbachia agariperforans* to accommodate strain KMM 3525^T. However, the prokaryotic genus name *Reichenbachia* Nedashkovskaya *et al.* 2003 is illegitimate because it is a later homonym of the plant genus name *Reichenbachia* Sprengel 1823 [Principle 2 of the Bacteriological Code (1990 Revision)] and a later homonym of the insect genus name *Reichenbachia* Leach 1825 [Principle 2 of the Bacteriological Code (1990 Revision)]. Consequently, we propose to replace the names *Reichenbachia* and *Reichenbachia agariperforans* with *Reichenbachiella* and *Reichenbachiella agariperforans*, respectively.

According to Nedashkovskaya *et al.* (2003), strain KMM 3525^T does not utilize carbohydrates, but the addition of sea water (50%) to the medium used for the sugar oxidation test revealed the formation of acid from L-arabinose and L-fucose. Testing of strain KMM 3525^T using the API 50 CH gallery (bioMérieux) also indicated acid formation from aesculin and arbutin. These findings are included in the description of *Reichenbachiella agariperforans*.

Description of *Pontibacter* gen. nov.

Pontibacter (Pon.ti.bac'ter. L. n. *pontus* the sea; N.L. masc. n. *bacter* from Gr. neut. n. *baktron* rod; N.L. masc. n. *Pontibacter* a marine bacterium).

Rod-shaped cells, motile by means of gliding. Gram-negative. Do not form endospores. Aerobic. Produce non-diffusible carotenoid pigments. Chemo-organotrophic. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The major respiratory quinone is MK-7. The main cellular fatty acids are straight-chain unsaturated and branched-chain unsaturated fatty acids C15:1 iso, C17:0 iso 3-OH, summed feature 3 (comprising C15:0 iso 2-OH and/or C16:1 ω 7) and summed feature 4 (consisting of

Table 2. Phenotypic characteristics that separate *Pontibacter actiniarum* KMM 6156^T and its close relatives belonging to the phylum 'Bacteroidetes'

Taxa: 1, *Pontibacter actiniarum* KMM 6156^T; 2, *Adhaeribacter aquaticus*; 3, *Hymenobacter*; 4, *Microscilla marina*; 5, *Algoriphagus*. Data from Reichenbach (1989), Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002), Bowman *et al.* (2003), Nedashkovskaya *et al.* (2004), Van Trappen *et al.* (2004), Rickard *et al.* (2005), Yoon *et al.* (2005b) and this study. M, Marine; T, terrestrial; A, air; FW, fresh water; P, pink; R, red; RP, red-pink; O, orange; OR, orange-red; V, variable; ND, not detected.

Characteristic	1	2	3	4	5
Habitat	M	FW	T, A	M	M
Cell size (µm)	0.3–0.4 × 1.2–1.9	0.9–1.7 × 2.8–4.1	0.4–1.0 × 1.3–5.0	0.5–0.6 × > 150	0.3–0.7 × 1.0–10.0
Gliding motility	+	–	–	+	–
Colony colour	P	P	RP, R	O	O, OR, P
α-Galactosidase	–	+	–	ND	+
β-Galactosidase	+	+	–	ND	+
Salinity range (%)	0–10	0–4	0–2	1–2	0–10
Temperature range (°C)	6–43	4–37	–0.5 to 42	35	–2 to 41
Hydrolysis of:					
Aesculin	+	ND	–	ND	+
Agar	+	–	–	–	V
Starch	–	+	+	–	V
Trypsin	+	ND	–	ND	+
Tween 80	–	ND	+	ND	V
DNA G + C content (mol%)	48.7	40.0	55–64	42.0	35–42

C17 : 1 iso I and/or C17 : 1 anteiso B). As determined by 16S rRNA gene sequence analysis, the genus *Pontibacter* is a member of the phylum 'Bacteroidetes'. The type species is *Pontibacter actiniarum*.

Description of *Pontibacter actiniarum* sp. nov.

Pontibacter actiniarum (ac.ti.ni.a'rum. N.L. gen. pl. n. *actiniarum* of sea anemones or related animals).

Displays the following properties in addition to those given in the genus description. Cells range from 0.3 to 0.4 µm in width and from 1.2 to 1.9 µm in length. On marine agar, colonies are circular, 2–3 mm diameter, convex, shiny, pink and smooth. Does not require Na⁺ or sea water for growth. Growth occurs at 6–43 °C; optimal temperature is 25–28 °C. Growth occurs at 0–10 % NaCl. No pigments of flexirubin type are formed. Decomposes agar (weakly), gelatin, DNA and Tweens 20 and 40. Does not hydrolyse casein, starch, Tween 80, cellulose (CM-cellulose or filter paper) or chitin. Forms acid from aesculin and arbutin (API 50 CH gallery; bioMérieux). Does not form acid from L-arabinose, D-cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, L-sorbose, D-sucrose, DL-xylose, N-acetylglucosamine, glycerol, adonitol, dulcitol, inositol or mannitol. Biolog GN2 tests show that the type strain utilizes dextrin, glycogen, methyl pyruvate, α-ketovaleic acid, DL-lactic acid, alaninamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic

acid, L-proline, methyl β-D-glucoside, α-ketobutyric acid, succinamic acid, L-alanyl-glycine, glycyl-L-aspartic acid and L-threonine. Does not utilize α-cyclodextrin, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, L-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-lactose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, monomethyl succinate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-, β- and γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, glucuronamide, D-alanine, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglyutamic acid, D-serine, L-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. Nitrate is not reduced. Indole, H₂S and acetoin (Voges-Proskauer reaction) production is negative. According to the API ZYM gallery (bioMérieux), produces β-galactosidase, acid phosphatase, esterase lipase (C8), leucine and valine arylamidases, trypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase and N-acetyl-β-glucosaminidase but not esterase (C4), lipase (C14),

cystine arylamidase, α -chymotrypsin, α -galactosidase, β -glucosidase, β -glucuronidase, α -mannosidase or α -fucosidase. The predominant fatty acids are C15:1 iso (28.8%), C17:0 iso 3-OH (6.5%), summed feature 3 (14.7%; comprising C15:0 iso 2-OH and/or C16:1 ω 7) and summed feature 4 (31.3%; consisting of C17:1 iso I and/or C17:1 anteiso B). Susceptible to ampicillin, benzylpenicillin, chloramphenicol, doxycycline, erythromycin, kanamycin, carbenicillin, lincomycin, oleandomycin and tetracycline. Resistant to gentamicin, neomycin, polymyxin B and streptomycin. The DNA G+C content is 48.7 mol%.

The type strain, KMM 6156^T (=KCTC 12367^T=LMG 23027^T), was isolated from unidentified actinians collected in Rudnaya Bay, Pacific Ocean.

**Description of *Reichenbachiella* gen. nov.
(previous illegitimate name *Reichenbachia*
Nedashkovskaya et al. 2003)**

Reichenbachiella (Rei.chen.bach.i.el'la. N.L. fem. dim. n. *Reichenbachiella* named in honour of Hans Reichenbach, a German microbiologist who has made a great contribution to the taxonomy of bacteria belonging to the phylum 'Bacteroidetes').

Rod-shaped, motile by gliding. Gram-negative. Do not form endospores. Aerobic. Produce non-diffusible carotenoid pigments. Chemo-organotrophic. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The major respiratory quinone is MK-7. The main cellular fatty acids are straight-chain unsaturated and branched-chain saturated fatty acids C15:0 iso, C16:1 ω 5c and summed feature 3 (comprising C15:0 iso 2-OH and/or 16:1 ω 7c). As determined by 16S rRNA gene sequence analysis, the genus *Reichenbachiella* is a member of the phylum 'Bacteroidetes'. The type species is *Reichenbachiella agariperforans*.

**Description of *Reichenbachiella agariperforans*
comb. nov. (illegitimate basonym
Reichenbachia agariperforans Nedashkovskaya
et al. 2003)**

Reichenbachiella agariperforans [a.ga.ri.per.fo'rans. N.L. n. *agarum* agar (algal polysaccharide); L. part. adj. *perforans* perforating (making holes); N.L. part. adj. *agariperforans* making holes in agar, bacterium making deep hollows in agar].

The description is as given by Nedashkovskaya *et al.* (2003) with the following additional characteristics. The type strain can form acid from L-arabinose, D-cellobiose, D-glucose and N-acetylglucosamine. According to the API 50 CH gallery (bioMérieux), it can form acid from arbutin and aesculin. Can produce leucine- and valine-arylamidases, trypsin, naphthol-AS-BI-phosphohydrolase, α - and β -galactosidases, α - and β -glucosidases, N-acetyl- β -glucosaminidase and alkaline and acid phosphatases, but not esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, α -chymotrypsin, β -glucuronidase, α -mannosidase or α -fucosidase. Flexirubin

pigments are produced. The predominant fatty acids are C15:0 iso (28.6%), C16:1 ω 5c (21.9%) and summed feature 3 (20.7%; comprising C15:0 iso 2-OH and/or C16:1 ω 7).

The type strain, KMM 3525^T (=NBRC 16625^T=JCM 11238^T), was isolated from sea water collected in Amursky Bay, Pacific Ocean.

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