

Puniceicoccus vermicola gen. nov., sp. nov., a novel marine bacterium, and description of *Puniceicoccaceae* fam. nov., *Puniceicoccales* ord. nov., *Opitutaceae* fam. nov., *Opitutaes* ord. nov. and *Opitutae* classis nov. in the phylum 'Verrucomicrobia'

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A Gram-negative, chemoheterotrophic, facultatively anaerobic coccus, designated IMCC1545^T, was isolated from the digestive tract of a marine clamworm, *Periserrula leucophryna*, inhabiting a tidal flat of the Yellow Sea. Cells of strain IMCC1545^T are non-motile, dividing by binary fission. The predominant fatty acids are anteiso-C_{15:0} and C_{18:0}. The respiratory quinone is menaquinone-7 and the DNA G+C content is 52.1 mol%. Phylogenetic analyses based on 16S rRNA gene sequences using three treeing algorithms revealed that the strain formed a novel genus-level lineage within the phylum 'Verrucomicrobia'. The most closely related named organisms to strain IMCC1545^T are '*Fucophilus fucoidanolyticus*' SI-1234 (86.5% 16S rRNA gene sequence similarity), *Alterococcus agarolyticus* ADT3^T (81.8%) and *Opitutus terrae* PB90-1^T (80.3%), which belong to subdivision 4 of the 'Verrucomicrobia'. Subdivision 4 of the 'Verrucomicrobia' (here named *Opitutae* classis nov.) was divided into two clades, a clade containing strain IMCC1545^T and a clade containing *Opitutus terrae*. From the taxonomic data obtained in this study, it is proposed that the new marine isolate be placed into a novel genus and species named *Puniceicoccus vermicola* gen. nov., sp. nov. (the type strain of *Puniceicoccus vermicola* is IMCC1545^T = KCCM 42343^T = NBRC 101964^T) within *Puniceicoccaceae* fam. nov and *Puniceicoccales* ord. nov in the class *Opitutae*. The family *Opitutaceae* fam. nov. and order *Opitutaes* ord. nov. are also formally proposed.

Although members of the phylum 'Verrucomicrobia' have shown to be very diverse in cultivation-independent surveys (Freitag & Prosser, 2003; Haukka *et al.*, 2005; Hugenholtz *et al.*, 1998; O'Farrell & Janssen, 1999), only a few members of the phylum have yet been cultivated (Chin *et al.*, 2001; Derrien *et al.*, 2004; Hedlund *et al.*, 1997; Hugenholtz *et al.*, 1998; Sangwan *et al.*, 2004; Schlesner, 1987; Ward-Rainey *et al.*, 1995). The phylum 'Verrucomicrobia' has been divided historically into five subdivisions, V1 to V5, based on 16S rRNA gene sequences (Hugenholtz *et al.*, 1998). In the second edition of *Bergey's Manual of Systematic Bacteriology* (Garrity *et al.*, 2003), however, three subdivisions are included at the rank of family: the families *Verrucomicrobiaceae* (subdivision 1), '*Xiphinematobacteriaceae*' (subdivision 2) and '*Opitutaceae*' (subdivision 4). Subdivision 1 includes the freshwater species *Verrucomicrobium spinosum*

(Schlesner, 1987), four freshwater species of the genus *Prostheco bacter* (Hedlund *et al.*, 1996, 1997) and the human-intestinal species *Akkermansia muciniphila* (Derrien *et al.*, 2004). Subdivision 2 contains only one cultured species, the recently identified '*Chthoniobacter flavus*' (Sangwan *et al.*, 2004). There are three identified species in subdivision 4, the soil-borne species *Opitutus terrae* (Chin *et al.*, 1999, 2001; Janssen *et al.*, 1997), *Alterococcus agarolyticus* (Shieh & Jean, 1998), isolated from a hot spring and originally misclassified within the class *Gammaproteobacteria*, and '*Fucophilus fucoidanolyticus*', isolated from a sea cucumber (Sakai *et al.*, 2003).

Here we report the characterization of a verrucomicrobial isolate cultured from the digestive tract of a marine clamworm. Taxonomic analyses indicated that this marine clamworm-associated isolate is distantly related to members of subdivision 4 of the phylum 'Verrucomicrobia' and represents a novel genus and species within subdivision 4. In addition, to clarify the taxonomic outline of subdivision 4 of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC1545^T is DQ539046.

the phylum 'Verrucomicrobia', we formally propose *Opitutae* classis nov., which is composed of two novel orders, *Puniceicoccales* ord. nov., containing *Puniceicocaceae* fam. nov., and *Opitiales* ord. nov., containing *Opitutaceae* fam. nov.

Benthic marine worms of the species *Periserrula leucophryna* (family Nereididae), a polychaete species endemic to the west coast of Korea, were collected in July 2005 from a depth of 1–2 m from tidal flat sediments on Donggum island (37° 35' 34.1" N 126° 31' 7.5" E). The marine worms were washed three times with sterile seawater and dissected under an Olympus SZH10 stereoscopic microscope. Entire invertebrate homogenates, digestive tract tissue homogenates and soil-like aggregates from within the digestive tract were used for isolating marine invertebrate-associated micro-organisms. One hundred microlitre aliquots of tissue homogenate were spread onto marine 2216 agar (MA; Difco) and the agar plates were incubated aerobically at 20 °C for 5, 14 or 30 days. A strain, designated strain IMCC1545^T, was initially isolated from a digestive tract tissue homogenate after incubation for 14 days. The strain was purified as single colonies on MA at 30 °C and was stored as 10% (v/v) glycerol suspensions at –80 °C. Unless otherwise indicated, cultures of the strain were grown routinely on MA at 30 °C.

Phenotypic characterizations were performed as described in previous studies (Cho & Giovannoni, 2003; Smibert & Krieg, 1994) using MA as the basal medium at 30 °C, unless otherwise noted. Cell morphology was examined by scanning electron microscopy (JSM 5410LV; JEOL), energy-filtering transmission electron microscopy (LIBLA120; Carl Zeiss) and phase-contrast microscopy (Nikon 80i; Nikon). Anaerobic growth was tested on MA at 30 °C using both the MGC anaerobic system and AnaeroPACK.Anaero (Mitsubishi Gas Chemical Company). Biochemical tests were carried out on API 20NE and API ZYM (bioMérieux) according to the manufacturer's instructions with artificial seawater (l⁻¹: 25.0 g NaCl, 1.0 g MgCl₂·6H₂O, 5.0 g MgSO₄·7H₂O, 0.7 g KCl, 0.15 g CaCl₂·2H₂O, 0.5 g NH₄Cl, 0.1 g KBr, 0.27 g KH₂PO₄, 0.04 g SrCl₂·6H₂O, 0.025 g H₃BO₃). Pigments of strain IMCC1545^T were extracted with acetone/methanol (1:1, v/v) and absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis). The ability of the strain to oxidize 95 different carbon sources was determined using Biolog GN2 microplates with artificial seawater. The following antibiotics were tested: ampicillin (10 µg), chloramphenicol (25 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), penicillin G (10 µg), rifampicin (50 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg).

Cells of strain IMCC1545^T are cocci, 0.6–1.0 µm in diameter (Fig. 1). No flagella were found. Carotenoid pigments with absorbance spectrum peaks at 320 and 480 nm were found; the latter peak was minutely separated into three peaks at 450, 480 and 504 nm. Anaerobic cultures grew much slower than aerobic cultures. In the API 20NE test, tests for nitrate

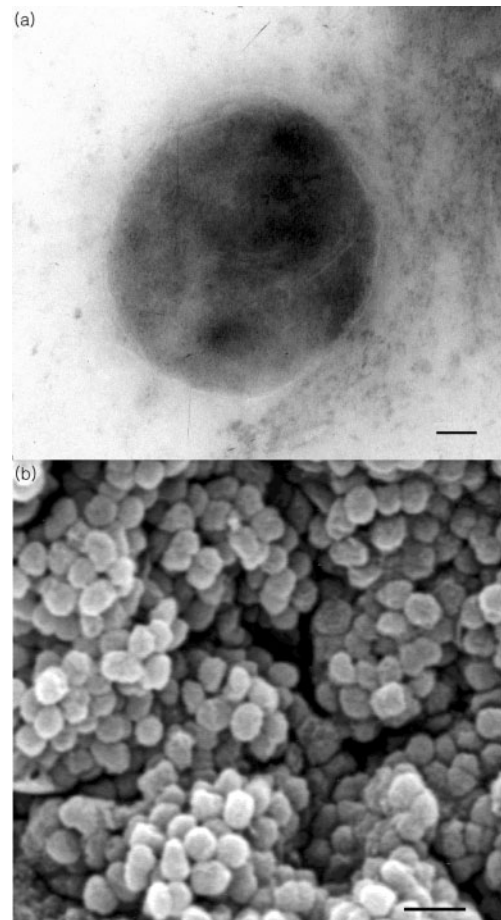


Fig. 1. Electron micrographs of strain IMCC1545^T. (a) Transmission electron micrograph of a negatively stained single cell. Bar, 0.1 µm. (b) Scanning electron micrograph of cell colonies. Bar, 1 µm.

reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, gelatinase and PNPG (β -galactosidase) were negative. In the API ZYM test, positive reactions were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative reactions were observed for lipase (C14), leucine, valine and cystine arylamidases, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. In tests with Biolog GN2 microplates, the strain utilized the following carbon compounds oxidatively: dextrin, D-arabitol, L-arabinose (weakly), D-cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, α -D-lactose, maltose, D-mannitol, D-mannose, methyl β -D-glucoside, D-psicose (weakly), L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester (weakly), succinic acid monomethyl ester, D-galactonic acid lactone, α -hydroxybutyric acid, α -ketobutyric acid (weakly), α -ketoglutaric acid, α -ketovaleric acid (weakly), DL-lactic

acid, succinic acid, succinamic acid (weakly), L-pyroglutamic acid, DL-carnitine (weak), 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. Strain IMCC1545^T did not utilize the remaining compounds of the GN2 microplate. The strain was resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, penicillin G, rifampicin, streptomycin and vancomycin and susceptible to erythromycin and tetracycline.

The DNA G + C content of strain IMCC1545^T was analysed by using HPLC with a Discovery C18 column (5 μ m, 15 cm \times 4.6 mm; Supelco) (Mesbah *et al.*, 1989). Respiratory quinones were analysed using reverse-phase HPLC by the Korea Culture Center of Micro-organisms (KCCM). Cellular fatty acid methyl esters were prepared from a culture grown on MA at 30 °C for 5 days and analysed according to the instructions of the Microbial Identification System (MIDI) by KCCM. The DNA G + C content of strain IMCC1545^T was 52.1 \pm 0.5 mol%. The only respiratory quinone detected was menaquinone-7. The major fatty acids in strain IMCC1545^T were anteiso-C_{15:0} (30.9%), C_{18:0} (24.7%), C_{16:0} (7.9%) and C_{17:0} (7.0%). Characteristics that differentiate strain IMCC1545^T from other members of subdivision 4 of the phylum 'Verrucomicrobia' are listed in Table 1.

A nearly full-length sequence of the 16S rRNA gene (1480 bp) of strain IMCC1545^T was obtained as described previously (Cho *et al.*, 2004) and used for phylogenetic and comparative sequence analyses. Preliminary BLASTN

network searches in GenBank showed that the strain belongs to the phylum 'Verrucomicrobia'. According to comparative 16S rRNA gene sequence analyses based on the BLASTN searches, the closest named species to strain IMCC1545^T were '*Fucophilus fucoidanolyticus*' (86.5% sequence similarity to the proposed type strain SI-1234), '*Alterococcus agarolyticus*' (81.8% similarity to strain ADT3^T) and '*Opiritatus terrae*' (80.3% similarity to strain PB90-1^T), and thus the strain was initially considered a novel member of the phylum 'Verrucomicrobia'. To clarify the phylogenetic position of strain IMCC1545^T, its 16S rRNA gene sequence was aligned carefully using the ARB package (Ludwig *et al.*, 2004) and only 1036 unambiguously aligned nucleotide positions were used for phylogenetic analyses in the ARB package and PAUP* version 4.0 beta 10 (Swofford, 2002). Neighbour-joining, maximum-parsimony and maximum-likelihood phylogenetic trees were generated as described previously (Cho *et al.*, 2004).

In all the phylogenetic trees generated, strain IMCC1545^T formed a tight clade together with the uncultured lake bacterium K2-S-20 within subdivision 4 of the 'Verrucomicrobia' (Fig. 2). This clade was strongly supported by high bootstrap values (100% in both neighbour-joining and maximum-parsimony trees) and clearly separated from the nearest clade containing '*Fucophilus fucoidanolyticus*', suggesting that strain IMCC1545^T should be placed in a novel genus and species. A large clade containing both '*Fucophilus fucoidanolyticus*' and strain IMCC1545^T, in spite of low bootstrap support, was clearly

Table 1. Characteristics that differentiate strain IMCC1545^T from other members of subdivision 4 of the 'Verrucomicrobia'

Strains: 1, IMCC1545^T; 2, '*Fucophilus fucoidanolyticus*' SI-1234 (Sakai *et al.*, 2003); 3, '*Alterococcus agarolyticus*' CCRC 19135^T (Shieh & Jean, 1998); 4, '*Opiritatus terrae*' DSM 11246^T (Chin *et al.*, 2001). +, Positive; -, negative; ND, no data available. Cells of all strains are cocci.

Characteristic	1	2	3	4
Source	Sea polychaete	Sea cucumber	Hot spring	Rice paddy soil
Cell size (μ m)	0.6–1.0	1.2–1.6	0.8–0.9	0.4–0.6
Oxygen demand	Facultatively anaerobic	Aerobic	Facultatively anaerobic	Obligately anaerobic
Flagella	–	–	+	+
DNA G + C content (mol%)	52.1 \pm 0.5	52	65.8	73.7 \pm 0.3
Quinone	Menaquinone-7	Menaquinone-7	ND	ND
NaCl concentration for growth (%)				
Range	1.0–10.0	1.0–7.5	1.0–3.5	ND
Optimum	2.5–3.0	3.0–3.5	2.0–2.5	3.0
pH for growth				
Range	5.5–9.5	5.0–12.0	7.0–8.5	5.5–9.0
Optimum	7.0–7.5	9.0	ND	7.5–8.0
Temperature for growth (°C)				
Range	10–40	8–37	40–56	10–37
Optimum	30–35	25–30	48	ND
Oxidase	–	–	+	–
Catalase	–	+	+	–
Major fatty acids (%)	anteiso-C _{15:0} (30.9), C _{18:0} (24.7), C _{16:0} (7.9)	ND	anteiso-C _{15:0} (51.5), C _{16:0} (10.6), C _{14:0} (6.4)	ND

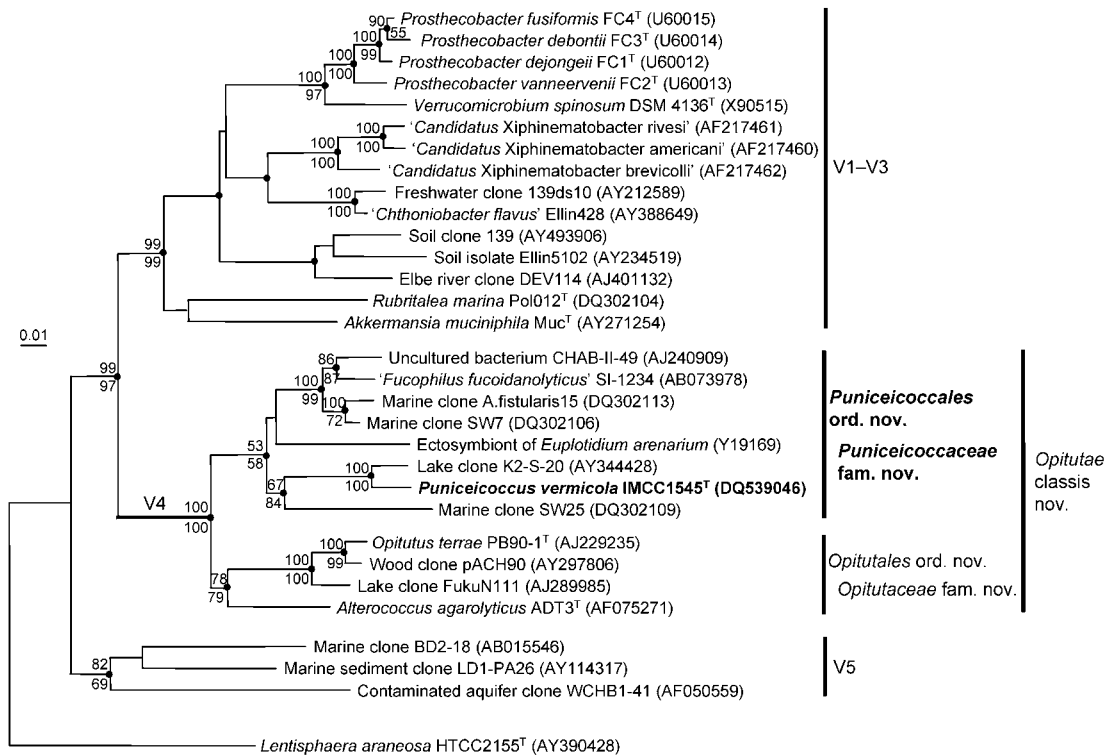


Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing relationships between strain IMCC1545^T and representatives of the phylum ‘*Verrucomicrobia*’. Bootstrap values (above 50 %) from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) are shown. Filled circles indicate nodes recovered reproducibly by all treeing methods. Other subdivisions of the phylum ‘*Verrucomicrobia*’ are shown. Bar, 0.01 substitutions per nucleotide position.

separated from a clade containing *Opitutus terrae*. Members of the large clade containing strain IMCC1545^T shared only 78.4–82.1 % 16S rRNA gene sequence similarity with members of the adjacent clade containing *Opitutus terrae*, suggesting that these two clades should be ranked as separate higher taxa, such as novel families or orders. Based on sequence information available from GenBank, the clade containing strain IMCC1545^T consists mainly of uncultured marine and aquatic bacteria and the clade containing *Opitutus terrae* consists largely of soil bacteria, indicating a habitat-specific distribution of members of the two clades.

In addition to phylogenetic differentiation of strain IMCC1545^T from the other members of subdivision 4 of the ‘*Verrucomicrobia*’, phenotypic characteristics clearly differentiated the strain from the most closely related species, ‘*Fucophilus fucoidanolyticus*’, *Alterococcus agarolyticus* and *Opitutus terrae* (Table 1). The G+C contents of strain IMCC1545^T and ‘*Fucophilus fucoidanolyticus*’ SI-1234 were 13.7–21.8 mol% lower than those of *Alterococcus agarolyticus* and *Opitutus terrae*, showing a distinct genetic difference between the two clades. Recently, the class ‘*Spartobacteria*’ and the order ‘*Chthoniobacterales*’ were proposed as new names equivalent to subdivision 2 of the

phylum ‘*Verrucomicrobia*’ (Sangwan *et al.*, 2004). Similar to the above proposal, we formally propose the name *Opitutae* classis nov. for subdivision 4 of the phylum ‘*Verrucomicrobia*’, which contains *Opitutales* ord. nov. and *Puniceicoccales* ord. nov. We also propose the names *Puniceicoccaceae* fam. nov. and *Opitutaceae* fam. nov. for the clades containing strain IMCC1545^T and the genus *Opitutus*, respectively, together with a novel genus and species, *Puniceicoccus vermicola* gen. nov., sp. nov., for strain IMCC1545^T.

Description of *Opitutae* classis nov.

Opitutae (O.pi.tu'tae. N.L. fem. pl. n. *Opitutales* type order of the class; -ae ending to denote a class; N.L. fem. pl. n. *Opitutae* the class of the order *Opitutales*).

Equivalent to subdivision 4 (Hugenholtz *et al.*, 1998) of the phylum ‘*Verrucomicrobia*’ and is defined by phylogenetic analyses based on 16S rRNA gene sequences obtained from four cultured representatives and a wide range of uncultured bacteria retrieved mainly from marine and soil habitats. Gram-negative. The class comprises the order *Opitutales* and the order *Puniceicoccales*. The type order is the order *Opitutales*.

Description of *Opiritales* ord. nov.

Opiritales (O.pi.tu.ta'les. N.L. masc. n. *Opiritus* type genus of the order; -ales ending to denote an order; N.L. fem. pl. n. *Opiritales* the order of the genus *Opiritus*).

Encompasses Gram-negative bacteria retrieved mainly from soil environments, within the class *Opiritae* of the phylum 'Verrucomicrobia'. The order contains the family *Opiritaceae*. Delineation of the order is determined primarily by phylogenetic information from 16S rRNA gene sequences. The type genus is the genus *Opiritus* (Chin *et al.*, 2001).

Description of *Puniceicoccales* ord. nov.

Puniceicoccales (Pu.ni.cei.coc.ca'les. N.L. masc. n. *Puniceicoccus* type genus of the order; -ales ending to denote an order; N.L. fem. pl. n. *Puniceicoccales* the order of the genus *Puniceicoccus*).

Encompasses Gram-negative bacteria retrieved mainly from marine environments, within the class *Opiritae*. The order contains the family *Puniceicoccaceae*. Delineation of the order is determined primarily by phylogenetic information from 16S rRNA gene sequences. The type genus is the genus *Puniceicoccus*.

Description of *Opiritaceae* fam. nov.

Opiritaceae (O.pi.tu.ta'ce.ae. N.L. masc. n. *Opiritus* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Opiritaceae* the family of the genus *Opiritus*).

Encompasses Gram-negative bacteria retrieved mainly from soil and terrestrial environments, including a hot spring and wood, within the order *Opiritales*. Currently, the family comprises the genera *Opiritus* and *Alterococcus*, together with several uncultured bacteria retrieved mainly from soil environments. Delineation of the family is determined primarily by phylogenetic information from 16S rRNA gene sequences. Gram-negative, motile, facultatively or obligately anaerobic cocci. The DNA G + C content of members of the family is 65–74 mol%. The type genus is the genus *Opiritus* (Chin *et al.*, 2001).

Description of *Puniceicoccaceae* fam. nov.

Puniceicoccaceae (Pu.ni.cei.coc.ca'ce.ae. N.L. masc. n. *Puniceicoccus* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Puniceicoccaceae* the family of the genus *Puniceicoccus*).

Encompasses Gram-negative bacteria retrieved mainly from marine and aquatic environments, within the order *Puniceicoccales*. Currently, the family comprises the genus *Puniceicoccus* and '*Fucophilus fucoidanolyticus*', together with several uncultured marine and lake bacteria. Delineation of the family is determined primarily by phylogenetic information from 16S rRNA gene sequences. The detailed description is the same as for the genus *Puniceicoccus*. The type genus is the genus *Puniceicoccus*.

Description of *Puniceicoccus* gen. nov.

Puniceicoccus (Pu.ni.cei.coc'cus. L. adj. *puniceus* pinkish red; N.L. masc. n. *coccus* from Gr. masc. n. *kokkos* a berry; N.L. masc. n. *Puniceicoccus* a pinkish-red-coloured coccus).

Cells are Gram-negative, non-motile, facultatively anaerobic cocci (oval-shaped, 0.6–1.0 µm in diameter). Carotenoid pigments are found. Require NaCl for growth. The predominant fatty acids are anteiso-C_{15:0} and C_{18:0}. The only respiratory quinone detected is menaquinone-7. The DNA G + C content is 52.1 ± 0.5 mol%. The type and only species of the genus is *Puniceicoccus vermicola*.

Description of *Puniceicoccus vermicola* sp. nov.

Puniceicoccus vermicola (ver.mi'co.la. L. n. *vermis* worm; L. suff. -cola from L. n. *incola* inhabitant; N.L. n. *vermicola* inhabitant of worms).

In addition to the characteristics reported for the genus, the following are added. Colonies on MA are uniformly circular, smooth, convex, opaque, pale-reddish coloured and 0.3–0.5 mm in diameter after 5 days of incubation. Colonies are up to 3 mm in diameter after 3 weeks of incubation. Growth occurs at 8–37 °C, optimally at 25–30 °C, but not at 4 or 42 °C. Growth occurs at pH 5–12 and 1.0–7.5% NaCl, occurring optimally at pH 9.0 and 3.0–3.5% NaCl. Oxidase and catalase are negative. Other phenotypic characteristics, including biochemical properties, carbon source utilization, enzyme activities and susceptibility to antibiotics, are given in the text. The fatty acids detected in the type strain are anteiso-C_{15:0} (30.9%), C_{18:0} (24.7%), C_{16:0} (7.9%), C_{17:0} (7.0%), iso-C_{14:0} (5.3%), C_{14:0} (4.9%), anteiso-C_{17:0} (3.6%), C_{18:0} 3-OH (2.5%) and C_{12:0} 3-OH (2.1%).

The type strain, IMCC1545^T (=KCCM 42343^T=NBRC 101964^T), was isolated from the digestive tract of a sea polychaete (*Periserrula leucophryna*) inhabiting a tidal flat of Donggum island, Korea.

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References

- Chin, K. J., Hahn, D., Hengstmann, U., Liesack, W. & Janssen, P. H. (1999). Characterization and identification of numerically abundant culturable bacteria from the anoxic bulk soil of rice paddy microcosms. *Appl Environ Microbiol* **65**, 5042–5049.
- Chin, K. J., Liesack, W. & Janssen, P. H. (2001). *Opiritus terrae* gen. nov., sp. nov., to accommodate novel strains of the division

- '*Verrucomicrobia*' isolated from rice paddy soil. *Int J Syst Evol Microbiol* **51**, 1965–1968.
- Cho, J. C. & Giovannoni, S. J. (2003).** *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the α -*Proteobacteria*. *Int J Syst Evol Microbiol* **53**, 1031–1036.
- Cho, J. C., Vergin, K. L., Morris, R. M. & Giovannoni, S. J. (2004).** *Lentisphaera araneosa* gen. nov., sp. nov., a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, *Lentisphaerae*. *Environ Microbiol* **6**, 611–621.
- Derrien, M., Vaughan, E. E., Plugge, C. M. & de Vos, W. M. (2004).** *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* **54**, 1469–1476.
- Freitag, T. E. & Prosser, J. I. (2003).** Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. *Appl Environ Microbiol* **69**, 1359–1371.
- Garrity, G. M., Bell, J. A. & Lilburn, T. G. (2003).** *Taxonomic outline of the prokaryotes*, release 4.0. New York: Springer.
- Haukka, K., Heikkinen, E., Kairesalo, T., Karjalainen, H. & Sivonen, K. (2005).** Effect of humic material on the bacterioplankton community composition in boreal lakes and mesocosms. *Environ Microbiol* **7**, 620–630.
- Hedlund, B. P., Gosink, J. J. & Staley, J. T. (1996).** Phylogeny of *Prostheco bacter*, the fusiform caulobacters: members of a recently discovered division of the bacteria. *Int J Syst Bacteriol* **46**, 960–966.
- Hedlund, B. P., Gosink, J. J. & Staley, J. T. (1997).** *Verrucomicrobia* div. nov., a new division of the bacteria containing three new species of *Prostheco bacter*. *Antonie van Leeuwenhoek* **72**, 29–38.
- Hugenholtz, P., Goebel, B. M. & Pace, N. R. (1998).** Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* **180**, 4765–4774.
- Janssen, P. H., Schuhmann, A., Morschel, E. & Rainey, F. A. (1997).** Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. *Appl Environ Microbiol* **63**, 1382–1388.
- 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.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- O'Farrell, K. A. & Janssen, P. H. (1999).** Detection of *Verrucomicrobia* in a pasture soil by PCR-mediated amplification of 16S rRNA genes. *Appl Environ Microbiol* **65**, 4280–4284.
- Sakai, T., Ishizuka, K. & Kato, I. (2003).** Isolation and characterization of a fucoidan-degrading marine bacterium. *Mar Biotechnol* **5**, 409–416.
- Sangwan, P., Chen, X., Hugenholtz, P. & Janssen, P. H. (2004).** *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, *Spartobacteria* classis nov., of the phylum *Verrucomicrobia*. *Appl Environ Microbiol* **70**, 5875–5881.
- Schlesner, H. (1987).** *Verrucomicrobium spinosum* gen. nov., sp. nov.; a fimbriated prosthecate bacterium. *Syst Appl Microbiol* **10**, 54–56.
- Shieh, W. Y. & Jean, W. D. (1998).** *Alterococcus agarolyticus*, gen. nov., sp. nov., a halophilic thermophilic bacterium capable of agar degradation. *Can J Microbiol* **44**, 637–645.
- Smibert, R. M. & Krieg, N. R. (1994).** *Phenotypic Characterization*. Washington, DC: American Society for Microbiology.
- Swofford, D. (2002).** PAUP* Phylogenetic Analysis Using Parsimony (and other methods). Sunderland, MA: Sinauer Associates.
- Ward-Rainey, N., Rainey, F. A., Schlesner, H. & Stackebrandt, E. (1995).** Assignment of hitherto unidentified 16S rDNA species to a main line of descent within the domain *Bacteria*. *Microbiology* **141**, 3247–3250.