Sulfuriroseicoccus oceanibius gen. nov., sp. nov., a representative of the phylum Verrucomicrobia with a special cytoplasmic membrane

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Abstract Here, we describe a novel bacterial strain, designated T37\(^\text{T}\), which was isolated from the marine sediment of Xiaoshi Island, PR China. Growth of strain T37\(^\text{T}\) occurs at 15–40 °C (optimum 37 °C), pH 6.0–9.0 (optimum 7.5), and in the presence of 0.5–5.5% (w/v) NaCl (optimum 1.5%). Characteristic biochemical traits of the novel strain include MK-9 as the major menaquinone. The major fatty acids identified were iso-C\(_{14}:0\) and C\(_{16}:1\) \(\omega 9c\) (oleic acid). Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphoglycolipids were the major cellular polar lipids. The G + C content of genomic DNA was 58.4 mol%. Unusual outer membrane features deduced from the analysis of cell morphology point towards the formation of an enlarged periplasmic space putatively used for the digestion of macromolecules. Phylogenetic analyses based on 16S rRNA genes and the genome indicated that strain T37\(^\text{T}\) represents a novel species and genus affiliated with a distinct family level lineage of the verrucomicrobial subdivision I. Our polyphasic taxonomy approach places the novel strain in a new genus within the current family Verrucomicrobiaceae, order Verrucomicrobiales, class Verrucomicrobiae. Strain T37\(^\text{T}\) (= KCTC 72799 \(^\text{T}\) = MCCC 1H00391 \(^\text{T}\)) is the type strain of a novel species, for which the name Sulfuriroseicoccus oceanibius gen. nov., sp. nov. is proposed.

Keywords Marine bacterium · Verrucomicrobiaceae · Uncommon membrane form · FtsZ

Abbreviations

PR People’s republic
KCTC Korean collection for type cultures
DSMZ Leibniz institute german collection of microorganisms and cell cultures
MCCC Marine culture collection of china
NBRC NITE biological resource center
BD Becton dickinson
MEGA Molecular evolutionary genetics analysis
MA Marine agar 2216
MB Marine broth 2216
OD Optical density
HPLC High-performance liquid chromatography
TLC Thin-layer chromatography
Introduction

Members of the phylum Verrucomicrobia play important roles in ecosystems, such as soil, seawater, freshwater, and even in the human gut. The phylum Verrucomicrobia was originally defined in 1997 (Hedlund et al. 1997). However, it was already recognised earlier by the application of 16S rRNA gene sequencing in 1987 (Albrecht et al. 1987). The PVC superphylum (Planctomycetes-Verrucomicrobia-Chlamydiae) was proposed in 2006 by Wagner and Horn. In a 16S rRNA-based phylogenetic tree, Planctomycetes, Verrucomicrobia, Chlamydiae and Lentisphaerae formed a monophyletic group together with the candidate phyla Poribacteria and OP3 (Wagner and Horn 2006). Verrucomicrobium spinosum was the first member with a sequenced genome (Sait et al. 2011), and a new bacterial division was suggested based on its distant phylogenetic position relative to other bacteria. Later, Hedlund established formal class-, order-, and family-level taxonomic categories. The phylum Verrucomicrobia has historically been divided into at least five subdivisions. Many current members of Verrucomicrobia are distantly related and even sufficient to belong to different phyla. One species in subdivision 5 has been proposed as the separate new bacterial phylum Kirimitatiellaeota (Spring et al. 2016), and Victivallis vadensis, originally regarded as a member of subdivision 7, is now known to be a member of the separate phylum Lentisphaerae (Cho et al. 2004).

Verrucomicrobia is highly diverse in cultivation-independent surveys based on the recovery of 16S rRNA gene sequences from many different habitats (Bergmann et al. 2011; Chiang et al. 2018). Members of the phylum have evolutionary and cellular significance regarding their relationship with specialized cell structures and an intriguing presence of close homologs to eukaryotic proteins, such as tubulins (Jenkins et al. 2003). Verrucomicrobia is also a medically important phylum, and includes species such as Akkermansia muciniphila, which may play key roles in microbial communities in humans (Depomnier et al. 2019). Additionally, members of the phylum are of immense significance to the understanding of ecology and their role in the global carbon cycle, such as members of the genus Methyloacidiphilum, which are important for the elucidation of bacterial methane oxidation (Hou et al. 2008).

Verrucomicrobia is a culturable representative of the unsampled but significant phylum. To date, only a few representatives have been cultivated, and more members of the phylum need to be brought into axenic culture and described. At the time of writing, only 60 species are effectively or validly described based on LPSN (List of Prokaryotic Names with Standing in Nomenclature) (Parte et al. 2020).

With regard to cell morphology, doughnut-shaped pits in the outer membrane turned out to be hallmark traits of members of the class Planctomycetia in the closely related phylum Planctomycetes, that also belongs to the PVC superphylum (Wiegand et al. 2020). It has been reported that the periplasmic space in members of the phylum Planctomycetes can be greatly enlarged and convoluted, possibly for macromolecule uptake (Boedeker et al. 2017).

Here, we present strain T37, that belongs to a novel genus and species within the phylum Verrucomicrobia. Doughnut-shaped pits were found in the outer membrane as a hallmark trait of this strain.

Material and methods

Isolation and cultivation

We considered that the cultivation of bacteria belonging to barely studied phyla is indeed possible when their ecological niche is sufficiently well mimicked. For this purpose, we designed “medium S” for cultures. The basal marine medium was designed in this study to match the salinity of seawater, which contained 80% (v/v) natural seawater, 20% (v/v) artificial seawater (consisting of 3.5% NaCl, 0.32% MgSO4, 0.22% MgCl2, 0.12% CaCl2, 0.07% KCl, 0.02% NaHCO3, and 1.0% Na2S2O3, each w/v), and 100 g/L fresh sediment. After filtering with gauze, filtrates were obtained. For providing carbon and nitrogen sources, the initial cultivation medium for marine bacterial strains was supplemented with 0.33 g/L sodium pyruvate, 0.5 g/L peptone (Hopebio), and 0.1 g/L yeast extract (Hopebio). The pH of the medium was adjusted to 7.5 with NaOH. All medium components were sterilised by autoclaving, except for a solution of NaHCO3, which was filtered through a 0.22 μm pore polyethersulfone filter. The isolates were obtained on solid media containing either 15 g/L agar (Hopebio). The solidifying agent
was autoclaved separately and added to the medium before pouring the plates.

Sediment samples were collected from Xiaoshi Island (37°31' 36" N, 122°00' 58" E), Weihai, PR China, which is a national marine nature reserve. The sediment sample was collected from approximately 10 cm depth in the intertidal zone after high tide in November 2018. The sample was serially diluted to 10⁻³ with sterilised seawater and 0.1 mL aliquots of each dilution were spread onto "medium S" plates. The plates were incubated in an aerobic environment for 30 d at 25 °C. After incubation, a tiny pink colony was selected from the plate. After repeated streaking, the strain was routinely cultivated on marine agar 2216 in a dilution of 1:10 (MA; BD) at 30 °C for 10–15 d.

The strain was stored in sterile 15% (v/v) glycerol supplemented with 1% (w/v) NaCl at −80 °C. The novel bacterium, designated T37 T, was isolated and selected for detailed taxonomic analyses, and was cultivated on MA at 37 °C unless stated otherwise.

Phylogeny based on 16S rRNA gene sequences

To classify the novel isolate, the 16S rRNA gene was amplified using primers 27F and 1492R (Lane 1991). Purified PCR products were sequenced by BGI Co., Ltd. (Qingdao, China), and a partial 16S rRNA gene sequence (1,511 bp) was obtained. The nearly full-length 16S rRNA gene sequence of strain T37 T was preliminarily identified by searching for matches in the EzBioCloud (http://www.ezbiocloud.net) and GenBank databases (https://www.ncbi.nlm.nih.gov) for further phylogenetic analysis. Comparative sequence analyses of the mostly complete 16S rRNA gene sequences were used to determine the phylogenetic position of strain T37 T.

To determine its exact taxonomic status, we performed additional phylogenetic analyses. The 16S rRNA gene sequences of the novel strain, all published verrucomicrobial strains and the uncultured strains, for which the 16S rRNA gene sequence similarities were higher than the 86.5% family threshold identity, were collected. Additionally, 16S rRNA gene sequences from genome sequences were extracted using RNAmer version 1.2 (Karin et al. 2007) and compared with the 16S rRNA gene reference database from phylogenetic trees through the analysis of BLAST sequence alignment to ensure authenticity. Next, according to similarity and completeness, duplicate 16S rRNA gene sequences were discarded from the study. Finally, the 16S rRNA sequences of 125 strains were obtained. Using MUSCLE (Edgar 2004), trimAl (Capella-Gutierrez et al. 2009) was used to automatically remove heterotypic sequences and differential regions according to the length and number of ambiguous bases. A comprehensive sequence alignment was generated using sequences extracted from genomic sequences or 16S rRNA gene sequences previously obtained from type strains. Based on the 16S rRNA gene sequence, a phylogenetic tree was reconstructed using FastTree 2 (Price et al. 2010) and RAxML version 8 (Alexandros 2014) and was visualised using MEGA version 7.0 (Kumars et al. 2016). A bootstrap analysis was performed with 1,000 replications to provide confidence estimates for tree topologies (Felsenstein 1985).

Genome sequencing, annotation, and analysis

The biomass of strain T37 T was collected, and genomic DNA was extracted according to the instructions of the genome extraction kit (TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Version 3.0). The extracted DNA was analysed by agarose gel electrophoresis for degradation and contamination, and a microspectrophotometer (Nanodrop, Thermo Fisher) was used to determine the extracted genomic DNA concentration to ensure an amount of at least 12 µg DNA. Using the extracted genomic DNA as a template for the PCR, the 16S rRNA gene sequence was amplified and sequenced, and the sequencing results were verified by BLAST comparison. After the bacterial strain information was confirmed, the extracted genomic DNA was sent to Tianjin Novozhiyuan Bio-Information Technology Co. Ltd. for genome sequencing. The PacBio platform and Illumina platform were used for library construction and inspection. After qualified library inspection, PacBio Sequel and Illumina NovaSeq PE150 were used for sequencing. Genome assembly was completed using SMRT Link Version 5.0.1 (Simon et al. 2018).

Protein-encoding genes were identified using the Cluster of orthologous group of proteins (COG) (Tatusov et al. 2003). The obtained genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline, and the genes involved in metabolic pathways were analysed in detail using information from the KEGG database (Kanehisa et al.
Secondary metabolite-associated gene clusters were predicted by antiSMASH 6.0 (Kai et al. 2021), carbohydrate-active enzymes were predicted by CAZy (Carbohydrate-Active enZYmes Database) (Cantarel et al. 2009), resistance genes were predicted by the Comprehensive antibiotic research database (CARD) (Alcock et al. 2019) and putative membrane transporter proteins were predicted by the Transporter classification database (TCDB) (Saier et al. 2006).

From the EzBioCloud (https://www.ezbiocloud.net) and NCBI databases (https://www.ncbi.nlm.nih.gov) all available genomes of published verrucomicrobial strains were downloaded. Then, the bacterial core gene set coverage was used to examine genome integrity, to determine whether the genome is polluted compared to the ContEst16S (Lee et al. 2017), and to retain genomes that have fewer than 500 contigs, a completeness of at least 95.0%, and no contamination. For the genome-based phylogenetic tree, UBCG (Na et al. 2018) was used to analyse the phylogenetic relationships. Based on 49 genomes, FastTree 2 (Price et al. 2010) using GTR + CAT parameters and IQtree (Jana et al. 2016) and the GTR + F + I + G4 model was used to construct the phylogenetic tree, and 1,000 bootstrap replicates were used for the analysis. The subsequent phylogenetic analysis was performed using MEGA version 7.0 (Kumars et al. 2016).

The genome sequences of characterized strains from the order Verrucomicrobiales were obtained from the NCBI database. Calculations of genomic G + C content values and genome size can also be considered valuable taxonomic marker (García-López et al. 2019). The genomic sequences of 22 strains were analysed, including the average amino acid identity (AAI) (Rodriguez and Konstantinidis 2014), percentage of conserved proteins (POCP) (Qin et al. 2014), average nucleotide identity (ANI) (http://jspecies.ribohost.com/jspeciesws/) (Yoon et al. 2017), and the digital DNA-DNA hybridisation (dDDH) (http://ggdc.dsmz.de/distcalc2.php) (Goris et al. 2007). However, we focused on the genetic relationships among different genera; therefore, we focused on analysing the AAI values based on the annotated genomes. The AAI values were calculated using Enveomics (http://enve-omics.ce.gatech.edu/aai/index), using both, best hits (one-way AAI) and reciprocal best hits (two-way AAI) between two genomic data-sets of proteins. The AAI values were set at a threshold of 60.0% as the boundary for the delineation of genera (Rodriguez and Konstantinidis 2014).

Cell morphological analyses

The determination of the cell characteristics of strain T37T was performed using cells grown on MB for 3 d at 37 °C. Cell division, morphology, and size were examined by light microscopy (Carl Zeiss Axioscope A1), scanning electron microscopy (model Nova NanoSEM450; FEI), and transmission electron microscopy (JEM1200, Japan).

Cells of strain T37T were collected and diluted 1:1000 (initial OD600 = 1.0), washed twice with 0.1 M phosphate-buffered saline (PBS) at pH 7.0, and resuspended in 200 μL staining solution FM4-64 (3 μg/mL), and the mixture was incubated at room temperature for 10 min. The cells were resuspended in 200 μL staining solution DAPI (4',6-diamidino-2-phenylindole):water (1:25), the final concentration of DAPI was 1 μg/mL, and the mixture was incubated at room temperature for 10 min. The cells were washed twice with PBS and centrifuged at 2,500 × g for 1.5 min. Finally, the cells were resuspended in 200 μL PBS. During the entire process, the cells were observed and photographed using a fluorescence microscope. The FM4-64 dye stains cell membrane lipids and emits red fluorescence (maximum excitation/emission wavelength is approximately 515/640 nm) (Amerik and Hochstrasser 2006), and DAPI is a fluorescent dye that strongly binds to DNA (Piotr et al. 2001). According to the intensity of fluorescence, the amount of DNA can be determined by the amount of blue light emitted (maximum excitation/emission wavelength is approximately 340/488 nm).

Transmission electron microscopy of ultrathin sections of strain T37T was performed as previously described (Pimentel-Elardo et al. 2003). For transmission electron microscopy, cells were grown in liquid culture and fixed with 2.5% (v/v) glutaraldehyde for 24 h at 4 °C, washed three times with PBS (0.1 M), pH 7.0. In the fume hood, the sample was fixed with 1% (v/v) osmium acid solution for 1–2 h, the osmium acid waste solution was carefully removed, and the sample was washed three times with 0.1 M PBS buffer at pH 7.0, 15 min each time. The samples were dehydrated with increasing concentrations of ethanol (from 30 to 100%, v/v) for 15 min at each
concentration and then treated with 100% (v/v) ethanol for 20 min. A drop of culture was incubated on a copper grid, the liquid was removed, and the cells were stained with a drop of 0.5% (w/v) uranyl acetate for 5 min. The cells were examined using transmission electron microscopy (JEM1200, Japan).

For scanning electron microscopy, colonies with surrounding material were fixed in 2.5% (v/v) glutaraldehyde for 4 h at 4 °C, washed three times with 0.1 M PBS, pH 7.0, and dehydrated with increasing concentrations of ethanol (from 30 to 100%, v/v) for 10 min. After critical-point drying and platinum coating of the dried material, the colonies were examined using scanning electron microscopy (model Nova NanoSEM450; FEI). Before photographing, it was important to select the field of view in which the cells did not overlap and block each other. Additionally, it was necessary to select a relatively high magnification to view single cells.

Physiological tests

The temperature range for growth was determined on MA at 4–45 °C. The NaCl concentrations for growth were determined by incubating the bacteria in modified marine broth 2216 made with 0.5% peptone, 0.1% yeast extract, and artificial seawater (0.32% MgSO4, 0.22% MgCl2, 0.12% CaCl2, 0.07% KCl, 0.02% NaHCO3, w/v) in the presence of 0–10% (w/v) NaCl at intervals of 0.5%. The influence of the pH was determined by adding the appropriate buffers (San-gon), including MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), and CAPSO (pH 9.0–9.5) to MB at a concentration of 20 mM, and the pH of the control groups was checked after autoclaving. The OD600 values of the cultures were measured after incubation for 3 d at 37 °C. Cells of strain T37T were obtained from cultures grown on MA at 37 °C for 3 d, and the following phenotypic tests were performed as described in previous studies (Feng et al. 2020). Motility was determined by the hanging-drop method, and gliding motility was tested by inoculating the bacteria on 0.5% agar. The Gram reaction was determined using the bioMérieux Gram-stain kit according to the manufacturer’s instructions. Growth under anaerobic conditions (10% H2, 5% CO2, and 85% N2) was determined after incubation for 14 d in an anaerobic chamber with or without 0.1% (w/v) KNO3. Susceptibility to antibiotics was investigated on MA using the disc diffusion method, and according to procedures outlined by the Clinical and Laboratory Standards Institute (Cockerill et al. 2011). Catalase activity was determined by observing bubble formation in a 3% (v/v) H2O2 solution. Oxidase activity was examined using an oxidase reagent kit (bioMérieux) according to the manufacturer’s instructions. Tests were performed for the hydrolysis of starch, casein, alginate, carboxymethylcellulose, and Tweens 20, 40, 60, and 80. All experiments were performed in triplicates. Biochemical tests were performed using API 50CHB (bioMérieux) kits (http://www.biomerieux-diagnostics.com/apir-id-strip-range) and API 20E (bioMérieux) kits according to the manufacturer’s instructions, using the biomass of strain T37T grown on MA at 37 °C for 3 d. Production of other enzymes was assessed using APIZYM kits (bioMérieux). Carbon source oxidation was checked using BIOLOG GEN III microplates (http://www.biolog.com/). All API tests were performed according to the manufacturer’s instructions, except that the salinity was adjusted to 1.5%. All API and BIOLOG tests were performed with two replicates.

Chemotaxonomic analysis

Biomass for the study of chemotaxonomic features of strain T37T was obtained from cultures grown in MB for 3 d (late exponential phase). Polar lipids were determined using 2D Thin-layer chromatography (TLC) (Minnikin et al. 1982). Four separate TLC plates (EMD Millipore, 1.16487.0001) were prepared for each sample and individually stained using phosphomolybdic acid solution (total lipids), molybdenum blue solution (phosphates), a-naphthol sulfuric solution (carbohydrates), and ninhydrin (amines); all reagents were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Isoprenoid quinones of strain T37T were analysed using reverse-phase HPLC (Kroppenstedt and Reiner 1982). The preparation and extraction of fatty acid methyl esters from biomass and their subsequent separation and identification by gas chromatography were performed as previously described (Athalye et al. 2010). The fatty acids were extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1), methylated, and analysed using an Agilent 6890 N gas chromatograph. Cellular fatty acids were identified...
using the TSBA40 database of the microbial identification system.

**Results**

**Isolation and identification of strain T37T**

To isolate novel strains of *Verrucomicrobia*, sediment samples were collected from Xiaoshi Island (37°31'36" N, 122°00'58" E), Weihai, PR China, which is a national marine nature reserve. “Medium S” was designed for cultivation and obtained colonies were screened by 16S rRNA gene sequencing analysis. A novel strain was identified as a member of the phylum *Verrucomicrobia* based on pairwise comparison of 16S rRNA gene sequences of species in the EzBioCloud and NCBI databases.

**Phylogenetic placement of strain T37T**

The 16S rRNA gene sequence of strain T37T was previously determined and deposited in the GenBank/EMBL/DDBJ database under accession number MN412654. Based on the pairwise comparison of 16S rRNA gene sequences of species in the EzBioCloud (http://ezbiocloud.net/eztaxon), strain T37T showed low levels of 16S rRNA gene sequence similarities to known members of the order *Verrucomicrobiales* and exhibited the highest sequence similarity with the uncultured strain SBYB-1201 (89.5%), followed by uncultured strain sediment_deep44 (89.2%), uncultured strain RESET_18A06 (88.9%), *Roseibacillus ponti* YM27-120 (89.0%), *R. persicicus* YM26-010 T (88.8%), and *R. ishigakijimensis* MN1-741 T (88.8%). The sequence of strain T37T showed a maximum identity of 89.0% within the cultured type strains of the *Verrucomicrobiaceae* family. The identity was higher than the 86.5% family threshold identity (Yarza et al. 2014). Following 16S rRNA-based taxonomic identity, the genus rank seems appropriate for this strain. To determine its exact taxonomic status, we performed further analyses. Phylogenetic analysis of the 16S rRNA gene sequences performed using MEGA version 7.0 showed that strain T37T formed a distinct lineage with the uncultured family GU230460 within the order *Verrucomicrobiales* (Fig. 1).

A phylogenetic analysis based on the genomic sequences performed with IQtree showed that strain T37T formed a distinct lineage within the order *Verrucomicrobiales* (Fig. 2), which supported that strain T37T could be assigned to a novel species of a novel genus. The degree of genomic similarity of strain T37T to closely related species was estimated using average amino acid identity (AAI) analysis. Values among closely related taxa ranged from 55.9% between strain T37T and the order *Verrucomicrobiales* to 58.3% (Fig. 3). When the isolate was compared to these close species, the highest value was 58.3% with *Rubritalea squalenifaciens* DSM 18772 T, which was below the genus threshold of 60.0%. Additionally, POCP, ANI, and dDDH were determined, and the values were lower than the threshold (POCP < 50.0% (genus threshold), ANI < 95.0% (species threshold), dDDH < 70.0%) (Table S1). Based on these results, the strain should be classified as a novel species of a novel genus.

**Genome characteristics and analysis of genome-encoded features**

The genome of strain T37T has a size of 3,496,488 bp with 58.4 mol% G + C content (Table 1). Sequencing of the genome yielded one contig. Of the 2,855 predicted genes, 2,772 were protein-coding genes, and 71 code for RNAs (5 copies each of 5S rRNA, 16S rRNA and 23S rRNA; 52 tRNAs, and four other RNAs). A total of 1,917 genes (69.2%) were assigned a putative function using NR BLAST. The remaining genes (n = 749) were annotated as hypothetical proteins (27.0% of the protein-coding genes). The properties and statistical information of the genome are presented in Table 1.

The distribution of genes into COG functional categories is shown in Fig. S1. The highest proportion of genes was assigned to the COG categories amino acid transport and metabolism [E], carbohydrate transport and metabolism [G], cell wall/membrane/envelope biogenesis [M], as well as translation, ribosomal structure, and biogenesis [J]. The proportion of genes in the mobilome: prophages and transposons [X] of strain T37T was higher than that of other strains (Fig. 4). Moreover, members of the PVC superphylum are usually specialized in the degradation of recalcitrant polysaccharides (Manuel et al. 2012). Carbohydrate-active enzymes were
predicted using the CAZy database, and high proportions of genes assigned to such catabolic enzymes (Fig. S2) probably belong to the class of glycoside hydrolases. The prediction of secondary metabolite gene clusters was performed using antiSMASH, and it was found that three gene clusters in strain T37T were putatively involved in the biosynthesis of secondary metabolites: two gene clusters belonged to terpene and one gene cluster belonged to type III polyketide synthase (T3PKS). However, the chemical structure of the secondary metabolites produced by enzymes encoded in these three gene clusters could not be inferred from the genome information. Strain T37T also harbours a type 2 secretion system (T2SS), two type 3 secretion systems (T3SS), and several putative prophages. Its chromosome harbours putative genes associated with known drug resistance to fluoroquinolone (mfd, gyrA), rifampicin (rpoB), isoniazid (katG), and efflux pumps conferring antibiotic resistance (abcA, adeCG, taeA). The genome of strain T37T contained approximately 125 membrane transport protein-related genes (Fig. S3).

Based on the annotated genome sequence, genes encoding proteins involved in the oxidative response (batA) and a major virulence factor (coproheme decarboxylase-HemQ) (Meuric et al. 2010) were found in the genome of strain T37T. It may be an important trait for cells of strain T37T to cope with toxic oxygen.

FtsZ is an almost universal tubulin homolog in bacteria (Addinall and Holland 2002; Vaughan et al. 2004). However, no ftsZ homolog was found in the genome of strain T37T. It was speculated that ftsZ in the genome of the strain T37T was not annotated because of the low similarity between the tubulin or ftsZ homologous sequences. It is also possible that, similar to members of the phylum Planctomycetes,
ftsZ is probably absent in strain T37^T (Wiegand et al. 2020).

The genes involved in metabolic pathways were analysed using information from the KEGG database. Sulphite reductase (NADPH-dependent) was found, and it was suggested that the addition of Na_2S_2O_3 to the isolation medium might be helpful for the isolation of strain T37^T.

Cell morphology and physiology

After 3 d of incubation at 37 °C on MA, colonies on MA were round, light pink, and glossy with entire margins. Cells of strain T37^T grew in various forms, including almost coccoid cells ranging from 0.5 to 1.0 μm in diameter and rod-shaped cells ranging from 0.4 to 0.9 μm in width and from 0.5 to 1.5 μm in length (Fig. 5a). Cells divide by binary fission. We speculated that the short rod-shaped cells could be in a state of cell division. To verify this hypothesis, the growth curve was determined, and cells of strain T37^T were observed at different growth periods. We found that in each period, coccoid and rod cells could be observed simultaneously (as shown in Fig. S4). Single cells and cell clusters were also identified. Motile cells, flagella, and prostheses were not observed. Cytoplasmic invaginations were observed in cells of strain T37^T (Fig. 5b–c, h). Additionally, differentiation within cells was observed in the thin sections, and the typical features of bacteria were visible, such as the plasma membrane (CM) and cell wall (Fig. 5f). Cells had major cytoplasmic intima (ICM) along their surface next to the cytoplasmic membrane (Fig. 5f). The cells had two major compartments. A larger compartment, between which the membrane was similar to the characteristics of the floating bacterium cell program, and a smaller endoplasmic compartment, with the cytoplasm located between the ICM and CM.

Based on a literature search, cells of some type strains of Verrucomicrobia also were coccoid or rod-shaped, such as Luteolibacter cuticulhirudinis E100^T (Glaeser et al. 2012), Rubritalea spongiae YM21-132^T, R. tangerina YM27-005^T (Yoon et al. 2007), R. marina DSM 17716^T (Scheuermann et al. 2006), and R. sabuli YM29-052^T (Yoon et al. 2008a, b). Generally, there are two main reasons for the change in bacterial cell shape from rod-shaped to coccoid.
Cells would change from rod-shaped to coccoid because they lack the MreB protein, such as the cells of *Bacillus subtilis* that change from rod-shaped to coccoid (Jones et al. 2001). It has been shown that the structural changes of penicillin-binding proteins (PBPs) caused by the functional defects of PBPs, can result in changes in cell morphology, especially the key control protein penicillin-binding protein 2 (PBP2) (Ogura et al. 1989). Only the genomic information of strain *R. marina* DSM 17716 T was obtained, and the genome was analysed. Genome analysis revealed that strain *R. marina* DSM 17716 T and strain T37 T only had the MreC protein and probably lacked the MreB protein. It was also possible that some cells of strain *R. marina* DSM 17716 T and strain T37 T were coccoid from short rod-shaped because of the deletion of or mutation in the *mreB* gene.

Doughnut-shaped pits were found in the outer membrane of *Gemmata obscuriglobus* (phylum Planctomycetes). A receptor-mediated protein uptake mechanism (Lonhienne et al. 2010) was proposed, suggesting that these bacteria might be able to perform...
Fig. 4 Comparison of the proportion of genes associated with the COGs functional categories of strain T37\textsuperscript{T} and other closely related species.

Fig. 5 Shape and ultrastructure of cells of strain T37\textsuperscript{T}. Cells were grown on MA at 37 ℃ for 3 days. Scanning electron microscopy and transmission electron micrograph of a negatively stained thin section of cells of strain T37\textsuperscript{T}. The selected micrographs originate from at least two independent experiments; more than 50 cells were analysed and the best representative image was chosen.
a simple form of endocytosis. However, the hypothesis of endocytosis is clearly refuted in a study by Boedeker et al. and the cytoplasmic membrane can give rise to an enlarged periplasmic space putatively used for the uptake and digestion of macromolecules (Boedeker et al. 2017). From the similarities between Planctomycetes and Verrucomicrobia, which belong to the PVC superphylum (Wagner and Horn 2006), it is speculated that the cytoplasmic membrane of strain T37T may be used for the digestion of macromolecules in a similar manner as proposed for planctomycetes. The most representative electron micrographs were selected from at least two independent experiments and more than 50 cells were analysed. The doughnut-shaped pits were indeed also found in the outer membrane of the cells of strain T37T (Fig. 5b–c, h). A series of pre-treatments was conducted before the bacterial cells were observed by electron microscopy, which might affect cell morphology. Therefore, the same pre-treatments were applied to strain T37T and Escherichia coli DH5α for scanning electron microscopy and transmission electron microscopy. FM4-64 and DAPI were used to stain the cells of E. coli DH5α and strain T37T, and fluorescence microscopy was used to observe the cells of E. coli DH5α and strain T37T after the same staining treatment. As a control, no pits appeared on the outer membrane of E. coli DH5α cells, and the DNA (shown in blue) of E. coli DH5α cells (Fig. 5e) was distributed evenly throughout the cells. From the fluorescence microscope image of T37T cells (Fig. 5d), the DNA clustered on one side of the cell, and the cell membrane of cells of strain T37T (shown in red) was not uniformly distributed. Based on the obtained results, it can be confirmed that the surface of strain T37T probably harbours pits.

Physiology

Strain T37T was Gram-stain-negative, facultatively anaerobic and non-motile. Colonies on MA were light pink. Growth occurred in presence of 0.5–5.5% (w/v) NaCl (optimum, 1.5%), at 15–40 °C (optimum, 37 °C), and at pH 6.0–9.0 (optimum, pH 7.5). Strain T37T reduced nitrate to nitrite. Cells of strain T37T were catalase-negative and oxidase-positive (weakly). The strain was positive for starch hydrolysis, but negative for hydrolysis of casein, Tweens 20, 40, 60 and 80, alginate, cellulose, and DNA. Strain T37T was susceptible to lincomycin (2 μg), chloramphenicol (30 μg), clarithromycin (15 μg), and erythromycin (15 μg), but resistant to carbenicillin (100 μg), penicillin (30 μg), ceftriaxone (30 μg), ampicillin (10 μg), rifampin (5 μg), cefotaxime sodium (30 μg), ofloxacin (5 μg), gentamycin (10 μg), tetracycline (30 μg), kanamycin (30 μg), tobramycin (10 μg), vancomycin (30 μg), neomycin (30 μg), norfloxacin (10 μg), and streptomycin (10 μg). PVC bacteria are generally believed to be susceptible to antibiotics that target protein biosynthesis, such as chloramphenicol, tetracycline, and erythromycin, or those that target DNA replication, such as fluoroquinolones (Cayrou et al. 2010; Godinho et al. 2019). However, the results are different from those previously reported, and strain T37T was resistant to ofloxacin and norfloxacin. The phenotypic characteristics determined by the API and BIOLOG tests are provided in the species description. Other phenotypic characteristics from classical experiments are listed in the species description, whereas the comparative analyses of phylogenetically related taxa are given in Table 2.

Chemotaxonomic characterizations

The menaquinone detected in strain T37T was MK-9. A list of the fatty acid composition of strain T37T is presented in Table 3. The major cellular fatty acids (> 10.0%) in strain T37T were iso-C14:0 (47.5%) and C16:1 ω9c (13.6%). C16:0 was not the main component that could be used to distinguish strain T37T from the closely related strains based on the type and proportion. The polar lipids consisted of phosphatidylethanolamine, phosphatidyglycerol, diphosphatidylglycerol, phosphoglycerolipids, phosphatidylcholine, phosphatidylinositol, one unidentified aminolipid, an unidentified phospholipid, and four unidentified lipids (Fig. S5). Strain T37T contained unsaturated fatty acids and branched-chain fatty acids as the major cellular lipids, and many kinds of phospholipids were identified as the major polar lipids.

Discussion and conclusion

The novel strain T37T was isolated from sediment and represents a novel genus that may be member of a novel family within the order Verrucomicrobiales. Characterisation of the first cultured representative of the Verrucomicrobia subdivision 1 revealed the
lifestyle of a facultatively anaerobic bacterium with cytoplasmic invaginations of the internal membrane. We speculate that the cytoplasmic membrane can form an enlarged periplasmic space putatively used for the digestion of macromolecules. It may constitute useful models for future experimental tests at the molecular level for how endogenous membrane invagination could give rise to endomembrane systems and the nucleus.

Furthermore, the here proposed genus *Sulfuriroseicoccus* has not been assigned to any definitive family within the order *Verrucomicrobiales*. The genus could be distinguished from genera in related families in the order *Verrucomicrobiales* by several features (Table 2). Unlike members of the family *Verrucomicrobiaceae*, strain T37T is not rod-shaped and is catalase-negative and oxidase-positive (weakly). In contrast to members of the genus *Rubritalea*, strain T37T could grow at 40 °C. Members of the genus *Akkermansia* are strictly anaerobic, but strain T37T is facultatively anaerobic. The four families could also be differentiated based on their chemotaxonomic features. Members of other families of the order *Verrucomicrobiales* have straight-chain fatty acids, unsaturated fatty acids, and branched-chain fatty acids as the major cellular lipids, whereas strain T37T does not have straight-chain fatty acids as the major cellular fatty acids. Additionally, strain T37T has phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphoglycolipids as the major polar lipids. Although the genus could be distinguished from genera in related families in the order *Verrucomicrobiales* by physiologic and chemotaxonomic characterizations, the clusters with members of *Verrucomicrobiaceae* in the phylogenetic trees (Figs. 1, 2) shows the current taxonomy of the order is highly chaotic. For the time being and given that major rearrangements are required to update the phylogeny of the order, we decided to assign the strain to a novel species of a novel genus and refrain from the introduction of a novel family.

**Description of sulfuriroseicoccus gen. nov.**

*Sulfuriroseicoccus* (Sul. fu.ri.ro.se.i.co.c’cus. L. neut. n. sulfur sulfur; L. masc. adj. roseus rosy, N.L. masc. n. coccus (from Gr. masc. n. kokkos a berry) a coccus; N.L. masc. n. *Sulfurisoreicoccus* rosy sulfur-reducing coccus).

Cells are Gram-stain-negative, facultatively anaerobic, cocci-shaped. Catalase-negative and oxidase-positive (weakly). The predominant menaquinone is

### Table 2 Characteristics that distinguish strain T37T from phylogenetically related taxa

| Characteristic           | 1                     | 2                     | 3                     | 4                     | 5                     |
|-------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Cell shape              | Coccoid or rod        | Rod                   | Rod                   | Oval                  | Coccoid or rod        |
| Colony colour           | Pink                  | Pink                  | Pale-pink             | White                 | Red                   |
| Tolerance to oxygen     | +                     | +                     | +                     | –                     | +                     |
| Oxidase                 | w                     | +                     | +                     | –                     | –                     |
| Catalase                | –                     | +                     | –                     | –                     | –                     |
| Temperature range (°C)  | 15–40                 | 20–37                 | 20–37                 | 20–40                 | 8–30                  |
| Growth pH               | 6.0–9.0               | 6.5–9.0               | 6.5–9.0               | 5.5–8.0               | 6.7–8.2               |
| Menaquinones            | MK-9                  | MK-9                  | MK-9                  | nd                    | MK-8, MK-9            |
| Major fatty acids (>10%)| *iso*-C<sub>14:0</sub>, C<sub>16:1</sub>ω9c | *iso*-C<sub>14:0</sub>, C<sub>16:1</sub>ω9c, C<sub>15:0</sub> | *iso*-C<sub>14:0</sub>, *anteiso*-C<sub>16:0</sub>, *anteiso*-C<sub>15:0</sub> | nd | *iso*-C<sub>14:0</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>ω7c and/or 2-OH *iso*-C<sub>15:0</sub> |
| DNA G + C content (mol%)| 58.4                  | 59.3                  | 60.9                  | 55.8                  | 51.6                  |

**Taxa:** 1, Strain T37T; 2, *Roseibacillus ishigakijimensis* KCTC 12986<sup>T</sup> (Yoon et al. 2008b); 3, *Haloferula rosea* KCTC 22201<sup>T</sup> (Yoon et al. 2008a); 4, *Akkermansia muciniphila* ATCC BAA-835<sup>T</sup> (Derrien et al. 2004); 5, *Rubritalea marina* DSM 17716<sup>T</sup> (Scheuermayer 2006). +, Positive; w, weakly positive; –, negative; nd, not determined
MK-9 and the major cellular polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphoglycolipids. The main cellular fatty acids are iso-C14:0 and C16:1ω9c. The genus belongs to the family Verrucomicrobiaceae, order Verrucomicrobiales, phylum Verrucomicrobia.

Description of sulfuriroseicoccus oceanibius sp. nov.

Sulfuriroseicoccus oceanibius (o.ce.a.ni’bi.us. L. masc. n. oceanus, the ocean; Gr. masc. n. bios, life; N.L. masc. n. oceanibius an ocean life).

Exhibits the following properties in addition to those given in the genus description. Cells are non-motile, non-flagellated, approximately 0.5–1.0 μm in diameter. Colonies on MA are round, light-pink and glossy with entire margins, about 1.0 mm in diameter after incubation for 3 days at 37 °C. Growth occurs at 15–40 °C (optimum, 37 °C), at pH 6.0–9.0 (optimum, 7.5) and in the presence of 0.5–5.5% (w/v) NaCl (optimum, 1.5%). Growth is not observed under anaerobic conditions on MA with or without 0.1% (w/v) KNO3. Nitrate is reduced to nitrite. Starch is hydrolyzed, but casein, Tweens 20, 40, 60, 80, alginate, cellulose and DNA are not. Positive for Voges–Proskauer reaction. The following substrates were oxidized: dextrin, d-trehalose, d-cellobiose, d-maltose, gentiobiose, α-d-lactose, sucrose, d-turanose, N-acetyl-β-d-mannosamine, N-acetyl-β-d-glucosamine, N-acetyl-d-galactosamine, d-mannose, d-fructose, l-fucose, l-rhamnose, l-galacturonic acid, l-galactonic acid lactone, glucuronamide, methyl pyruvate, d-lactic acid methyl ester, d-malic acid, l-malic acid and α-keto-butyric acid. Positive for tryptophan deaminase, gelatinase, alkaline phosphatase, esterase (C4), leucine arylamidase, N-acetyl-β-glucosaminidase, acid phosphatase, naphthol-AS-BI-phosphphydrolase, but negative for esterase lipase (C8), lipase (C14), cystine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, β-mannosidase and β-fucosidase. Sensitive to lincomycin, chloramphenicol, clarithromycin and erythromycin, but resistant to carbenicillin, penicillin, ceftriaxone, ampicillin, rifampin, cefotaxime sodium, ofloxacin, gentamycin, tetracycline, kanamycin, tobramycin, vancomycin, neomycin, norfloxacin and streptomycin.

The type strain is T37T (= KCTC 72799T = MCCC 1H00391T), isolated from the marine sediment of Xiaoshi Island, Weihai, PR China. The complete genome of strain T37T was determined to be 3.5 Mbp in size and the DNA G + C content of the type strain is 58.4 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain T37T is MN412654 and for the genome sequence is CP066776.1.

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Author contributions XF performed most of the experiments, wrote the main part of the manuscript, and functions as the first author. QHZ helped with the isolation of the novel strain and with cultivation measurements. MQY contributed to the literature search and analysed the sequencing data. XF and ZJD designed the study and helped with experimental setups and design.

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Table 3 Fatty acid composition of strain T37T

| Lipid type                | Abundance (%) |
|---------------------------|---------------|
| Branched fatty acids      |               |
| iso-C14:0                 | 47.5          |
| anteiso-C15:0             | 4.2           |
| iso-C16:0                 | 9.4           |
| Unsaturated fatty acids   |               |
| C15: 10ω8c                | 6.8           |
| C16: 10ω9c                | 13.6          |
| C17: 10ω8c                | 1.5           |
| C18: 10ω9c                | tr            |
| Straight-chain fatty acids|               |
| C14: 0                    | 3.3           |
| C16: 0                    | 5.4           |
| C17: 0                    | 1.7           |
| C18: 0                    | tr            |

Only fatty acids comprising ≥ 1.0% of the total are shown. Tr, fatty acids amounting (< 1.0%) are not shown. Major components (≥ 10.0%) are given in bold typeface.
Data availability The 16S rRNA gene sequence and the complete genome sequence of strain T37, are deposited in GenBank under accession numbers MN412654 and CP006677.1, respectively. The type strain T37 can be obtained from the Korean Collection for Type Cultures (KCTC 72799T) and the Marine Culture Collection of China (MCCC 1H00391T).

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships.

References

Addinall SG, Holland B (2002) The tubulin ancesstor, FtsZ, draughtsman, designer and driving force for bacterial cytokinesis. J Mol Biol 318:219–236
Albrecht W, Fischer A, Smida J, Stackebrandt E (1987) Verrucovibrio spinosum, a eubacterium representing an ancient line of descent. Syst Appl Microbiol 10:57–62
Alcock BP, Raphenya AR, Lau T, Kara KT, Mégane B et al (2019) CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525
Alexandros S (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 31:12–131
Amerik A, Hochstrasser SM (2006) A conserved late endosome-targeting signal required for Doa4 deubiquitylating enzyme function. J Cell Biol 175:825–836
Athalye M, Noble WC, Minnikin DE (2010) Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. J Appl Bacteriol 58:507–512
Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA et al (2011) The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biol Biochem 43:1450–1455
Boedeker C, Schüler M, Reinjes G, Jeske O, van Teeseling MCF et al (2017) Determining the bacterial cell biology of Planctomycetes. Nat Commun 8:14835
Cantarel BL, Coutinho PM, Corinle R et al (2009) The Carbohydrate-Active EnZymes database (CAZY): an expert resource for Glycogenomics. Nucleic Acids Res 37:D233–D238
Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973
Cayrou C, Raoult D, Drancourt M (2010) Broad-spectrum antibiotic resistance of Planctomycetes organisms determined by Etests. J Antimicrob Chemother 65:219–222
Chiang E, Schmidt ML, Berry MA, Biddanda BA, Burtner A, Johengen TH et al (2018) Verrucomicrobia are prevalent in north-temperate freshwater lakes and display class-level preferences between lake habitats. Plos One 13(3):e0195112
Cho J, Vergin KL, Morris RM, Giovannoni SJ (2004) Lentisphaera araneosa gen. nov., sp. nov, a transparent exopolymer-producing marine bacterium, and the description of a novel bacterial phylum. Lentisphaeraceae. Environ Microbiol 6:611–621
Cockerill F, Wikler M, Bush K, Cockerill FR (2011) Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement: CLSI document M100-S21
Depommier C, Everard A, Druart C, Plovier H, Hul MV et al (2019) Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med 25:1096
Derrien M, Vaughan EE, Plugge CM, de Vos WM (2004) Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 54:1469–1476
Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Feng X, Zou QH, Zhang XY, Ye MQ, Du ZJ (2020) Oceanicultrichito cocoides gen. nov., sp. nov., isolated from marine sediment within the family Puniceicocaceae. Int J Syst Evol Microbiol 70(11):5654–5664
Garcia-López M, Meier-Kolthoff JP, Tindall BJ, Gronow S, Woyke T et al (2019) Analysis of 1,000 type-strain genomes improves taxonomic classification of Bacteroidetes. Front Microbiol 10:2083
Glaeser SP, Galalis H, Martin K, Kämpfer P (2012) Luteolibacter cuticulihirudinis sp. nov., isolated from Hirudo medicinalis. Antonie Van Leeuwenhoek 102:319–324
Godinho O, Calisto R, Øvrea˚s L, Quinteira S, Lage OM (2019) Antibiotic susceptibility of marine Planctomycetes. Antonie Van Leeuwenhoek 112:1273–1280
Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Van-Damme P et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91
Hedlund BP, Gosink JJ, Staﬂey JT (1997) Verrucomicrobia div. nov., a new division of the bacteria containing three new species of Prosthecobacter. Antonie Van Leeuwenhoek 72:29–38
Hou S, Makarova KS, Saw JH, Senin P, Ly V, BV, et al (2008) 13(3):e0195112
Cho J, Vergin KL, Morris RM, Giovannoni SJ (2004) Lentisphaera araneosa gen. nov., sp. nov, a transparent exopolymer-producing marine bacterium, and the description of a novel bacterial phylum. Lentisphaeraceae. Environ Microbiol 6:611–621
Cockerill F, Wikler M, Bush K, Cockerill FR (2011) Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement: CLSI document M100-S21
Depommier C, Everard A, Druart C, Plovier H, Hul MV et al (2019) Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med 25:1096
Derrien M, Vaughan EE, Plugge CM, de Vos WM (2004) Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 54:1469–1476
Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:113
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Feng X, Zou QH, Zhang XY, Ye MQ, Du ZJ (2020) Oceanicultrichito cocoides gen. nov., sp. nov., isolated from marine sediment within the family Puniceicocaceae. Int J Syst Evol Microbiol 70(11):5654–5664
Garcia-López M, Meier-Kolthoff JP, Tindall BJ, Gronow S, Woyke T et al (2019) Analysis of 1,000 type-strain genomes improves taxonomic classification of Bacteroidetes. Front Microbiol 10:2083
Glaeser SP, Galalis H, Martin K, Kämpfer P (2012) Luteolibacter cuticulihirudinis sp. nov., isolated from Hirudo medicinalis. Antonie Van Leeuwenhoek 102:319–324
Godinho O, Calisto R, Øvrea˚s L, Quinteira S, Lage OM (2019) Antibiotic susceptibility of marine Planctomycetes. Antonie Van Leeuwenhoek 112:1273–1280
Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Van-Damme P et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91
Hedlund BP, Gosink JJ, Staﬂey JT (1997) Verrucomicrobia div. nov., a new division of the bacteria containing three new species of Prosthecobacter. Antonie Van Leeuwenhoek 72:29–38
Hou S, Makarova KS, Saw JH, Senin P, Ly V, BV, et al (2008) Complete genome sequence of the extremely acidophilic methanotroph isolate V4, Methylacidiphilum infernorum, a representative of the bacterial phylum Verrucomicrobia. Biol Direct 3:26
Jana T, Lam-Tung N, Arndt von H, Quang MB (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum like-lihood analysis. Nucleic Acids Res W232–W235
Jenkins C, Samudrala R, Anderson I, Hedlund BP, Petroni G et al (2003) Genes for the cytoskeletal protein tubulin in the bacterial genus Prosthecobacter. Proc Natl Acad Sci 99:17049–17054
Jones LJ, Carballido-López R, Errington J (2001) Control of cell shape in bacteria: helical, actin-like filaments in Bacillus subtilis. Cell 104:913–922
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. Int J Syst Evol Microbiol 58:998–1007

Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286

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