Ignavibacterium album gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of *Ignavibacteria* classis nov., for a novel lineage at the periphery of green sulfur bacteria

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A moderately thermophilic chemoheterotrophic bacterium, strain Mat9-16^T, was isolated from microbial mats developed in hot spring water streams from Yumata, Nagano, Japan. Cells of strain Mat9-16^T were strictly anaerobic, Gram-stain-negative, non-sporulating, non-motile and short to long rods (2.0–15.5 µm in length). Strain Mat9-16^T grew fermentatively with optimum growth at 45 °C, pH 7.0–7.5 and 1 % NaCl (w/v). Phylogenetic analysis based on the 16S rRNA gene revealed that strain Mat9-16^T was affiliated with an uncultivated lineage, and the nearest cultivated neighbours were green sulfur bacteria belonging to the class *Chlorobea* with 77–83 % sequence similarity. However, strain Mat9-16^T could not grow phototrophically and did not possess light-harvesting structures, morphologically and genetically, such as the chlorosomes of green sulfur bacteria. On the basis of phenotypic features and phylogenetic position, a novel genus and species are proposed for strain Mat9-16^T, to be named *Ignavibacterium album* gen. nov., sp. nov. (=NBRC 101810^T =DSM 19864^T). We also propose to place the cultivated bacterial lineage accommodating the sole representative Mat9-16^T in a novel class, *Ignavibacteria* classis nov. In addition, we present a formal description of the phylum-level taxon 'Chlorobi' as *Chlorobi* phyl. nov.

Microbial diversity in nature has been investigated phylogenetically by both culture-dependent and -independent approaches, and it is obvious that the majority of micro-organisms have not yet been cultivated in synthetic laboratory media (Amann *et al.*, 1995; Barns *et al.*, 1994; Hugenholtz *et al.*, 1998; Olsen *et al.*, 1986; Pace *et al.*, 1986; Ward *et al.*, 1990). Even in terrestrial hot springs in Japan, various culture-independent approaches, such as 16S rRNA gene-based phylogenetic analysis and quinone profile analysis, have revealed that uncultivated bacteria belonging to the so-called 'sulfur-turf bacteria', the genera

Thermoanaerobacterium and *Chloroflexus*, the cyanobacteria and the purple phototrophic bacteria occur in microbial mats and streamers (Hiraishi *et al.*, 1999; Nakagawa & Fukui, 2002, 2003; Yamamoto *et al.*, 1998).

On the other hand, various kinds of rare and/or unique micro-organisms have been isolated from terrestrial hot springs in Japan: e.g. *Caldilinea aerophila*, the filamentous thermophilic bacterium of subphylum I belonging to the phylum *Chloroflexi* (Sekiguchi *et al.*, 2003); *Calditerrivibrio nitroreducens*, the thermophilic nitrate-reducing bacterium belonging to the phylum *Deferribacteres* with a single order and family (Iino *et al.*, 2008); *Roseiflexus castenholzii*, the filamentous photosynthetic bacterium lacking chlorosomes (Hanada *et al.*, 2002); *Vulcanisaeta distributa*, the hyper-thermophilic and acidophilic sulfate-reducing archaeon belonging to the phylum *Crenarchaeota* (Itoh *et al.*, 2002); the thermophilic bacterium affiliated with the candidate phylum OP5 (Mori *et al.*, 2008). These facts predict that novel micro-organisms with a high level of diversity inhabit the terrestrial hot springs in Japan.

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Mat9-16^T is AB478415.

This paper describes the isolation of an anaerobic bacterium, strain Mat9-16^T, from the microbial mats developed in streams of hot spring water at Yumata, Japan, where the microbial diversity has been studied previously (Hiraishi *et al.*, 1999; Nakagawa & Fukui, 2003). On the basis of morphological, biochemical, physiological, chemotaxonomic and phylogenetic properties, a novel genus and species are proposed for this bacterium. In addition, we also propose a novel class for the bacterial lineage accommodating this bacterium, and formally describe the phylum-level taxon '*Chlorobi*' as *Chlorobi* phyl. nov.

Green- and yellow-coloured microbial mats were collected from a sulfide-rich hot spring at Yumata, Nagano, Japan (36° 23' 10" N 137° 45' 40" E). The microbial mats developed in the streams at a temperature of 37 °C. The collected samples were transported to our laboratory in a sealed nylon bag with an O₂-absorbing and a CO₂-generating agent (Anaero-Pack; Mitsubishi Gas Chemical).

The basal medium used, designated S medium, was composed of 0.355 g KCl, 0.14 g KH₂PO₄, 0.14 g CaCl₂·2H₂O, 0.25 g NH₄Cl, 4.0 g MgCl₂·6H₂O, 3.45 g MgSO₄·7H₂O, 6.0 g NaCl, 2.0 mg Fe(NH₄)₂(SO₄)₂·6H₂O, 2.0 g yeast extract (Becton Dickinson), 2.0 g trypticase peptone (BBL), 5.0 g NaHCO₃ and 10.0 ml trace elements solution (Iino *et al.*, 2009) per litre distilled water. Prior to inoculation, the pH of the medium was adjusted to 7.0 with 6 M HCl, dissolved air was removed by flushing with N₂/CO₂ (4:1, v/v), and 10 ml vitamin solution l⁻¹ (Wolin *et al.*, 1963) and 10 ml Na₂S/cystain-HCl solution l⁻¹ (Iino *et al.*, 2009) were added.

Approximately 1 g wet weight of the microbial mats was used for isolation of bacteria. The microbial mats were homogenized mechanically and suspended in 0.85 % saline. Serial decimal dilutions (10⁻¹ to 10⁻¹⁰) of the suspension were made with saline, 0.1 ml of the diluted samples were spread on S agar (1.5 %, w/v) plates, and cultivated for three months or more at 37 °C in a sealed nylon bag with an O₂-absorbing and CO₂-generating agent. Visible colonies grown on agar plates were picked up and transferred to vials containing fresh GS medium: S medium in which 10 mM D-glucose was added while flushing the medium with N₂/CO₂ (4:1, v/v). The vials were subsequently sealed with tight-fitting butyl rubber stoppers and incubated at 37 °C for one month. The cultures were further purified anaerobically on slants of GS medium solidified with 1.5 % (w/v) agar. The purification procedure was repeated several times until the cultures were deemed pure, and a uniformly shaped axenic culture, designated Mat9-16^T, was obtained. After purification, the isolate was maintained in GS medium while flushing with N₂/CO₂ (4:1, v/v). *Chlorobaculum tepidum* NBRC 103806^T used as a reference strain was cultivated in NBRC medium no. 855 (<http://www.nbrc.nite.go.jp/>).

Cells of strain Mat9-16^T were short to long rods and approximately 2.0–15.5 µm long and 0.2–0.3 µm wide (Fig. 1a, b). Motility and spore formation were not observed under a phase-contrast microscope. Flagellation was also not observed by electron microscopy. Ultrathin sections of whole cells of strain Mat9-16^T revealed a cytoplasmic membrane surrounded by a surface layer (Fig. 1c, d). Strain Mat9-16^T did not contain chlorosomes, which are the light-harvesting structures of green sulfur bacteria. Cells of strain Mat9-16^T were colourless and stained Gram-negative by conventional Gram staining.

Fig. 1. Phase-contrast micrograph (a) and transmission-electron micrographs (b–d) of cells of strain Mat9-16^T. Negatively stained cells and ultrathin sections of the cells are shown in (b) and (c, d), respectively. Bars, 5 µm (a), 2 µm (b), 0.5 µm (c) and 0.1 µm (d).

Strain Mat9-16^T was a strict anaerobe capable of growing under a N₂/CO₂ (4:1, v/v) atmosphere, but could not grow under microaerobic or aerobic conditions. Strain Mat9-16^T could not grow in S medium under incandescent light (500 lx). In addition, 0.01–0.5% yeast extract (Difco) was required for growth of the strain, but 1.0% yeast extract inhibited its growth. Catalase and oxidase reactions were negative. The growth temperature for strain Mat9-16^T ranged from 30 to 55 °C, with an optimum of 45 °C. No growth was observed at 25 or 60 °C. The pH range for growth was 6.5–8.0, with an optimum pH range being 7.0 to 7.5. No growth was observed at pH 5.5 or 8.5. Growth occurred below 3% (w/v) NaCl, with the optimum being 1% (w/v) NaCl. No growth was observed at 4% (w/v) NaCl. Strain Mat9-16^T grew fermentatively on D-glucose, D-mannose, D-fructose, maltose, cellobiose, D-salicin and pyruvate (all at 10 mM). No growth occurred on L-arabinose, D-ribose, D-xylose, D-galactose, D-sorbose, rhamnose, melibiose, trehalose, lactose, sucrose, raffinose, melezitose, D-sorbitol, D-mannitol, *myo*-inositol, formate, acetate, propionate, lactate, *n*-butyrate, fumarate, succinate, malate, citrate, gluconate, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol or 2-butanol (all at 10 mM). Elemental sulfur (1%, w/v), sulfate (10 mM), thiosulfate (10 mM), sulfite (1 mM), nitrate (10 mM), nitrite (1 mM), Fe(III) oxide (10 mM), Fe(III) citrate (10 mM), Mn(IV) oxide (10 mM), selenate (1 mM), selenite (1 mM), arsenate (1 mM), arsenite (1 mM), fumarate (10 mM) and oxygen [5% (v/v) in N₂] were not utilized as alternative electron acceptors in the presence of yeast extract. Strain Mat9-16^T was susceptible to chloramphenicol, rifampicin, tetracycline and vancomycin (all at 100 µg ml⁻¹), weakly susceptible to bacitracin and gentamicin (both at 100 µg ml⁻¹), but resistant to ampicillin, kanamycin and streptomycin (all at 100 µg ml⁻¹). The generation time was calculated to be 78.8 h in GS medium at 45 °C, pH 7.0 and 1% (w/v) NaCl, based on the increase in turbidity.

The major cellular fatty acids were anteiso-C_{15:0} (33.9%) and iso-C_{15:0} (28.7%), as determined by using the MIDI microbial identification system (Microbial ID; Agilent Technologies) based on the method described by Sasser (1990). C_{15:0} (4.0%), C_{16:0} (6.5%), iso-C_{17:0} (7.5%) and anteiso-C_{17:0} (3.8%) were also detected as minor components. The major isoprenoid quinone was identified as menaquinone-7 (MK-7), determined by using the HPLC method described by Komagata & Suzuki (1987). The genomic DNA G+C content of strain Mat9-16^T was 33.5 mol%, determined by the HPLC method described by Tamaoka & Komagata (1984).

The 16S rRNA gene of strain Mat9-16^T was amplified by PCR, and an almost-complete 16S rRNA gene sequence (1461 bases) was determined as described previously (Iino *et al.*, 2007). The 16S rRNA gene sequence of strain Mat9-16^T showed similarities of less than 83% with any of the known cultivated bacteria. The highest sequence similarity was shared with the following two environmental clones:

clone LS4-176 (Genbank accession no. AB234252) from the microcosms constructed using sediment mud and soil slurry contaminated with polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) (93.6% similarity; Hiraishi *et al.*, 2005) and clone 655939 (DQ404811) from soils contaminated with nitrate and heavy metals (93.0% similarity; Abulencia *et al.*, 2006). The nearest cultivated neighbours of this strain were the green sulfur bacteria affiliated with the class *Chlorobea*, with sequence similarities ranging from 77.4 to 82.7%. For the phylogenetic analysis, a bacterial domain reference sequence dataset described by Hugenholtz (2002) was used. The almost complete 16S rRNA gene sequences related to strain Mat9-16^T (more than 1300 nucleotides) were obtained from public databases (NCBI, <http://www.ncbi.nlm.nih.gov/>; greengenes, <http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>; SILVA, <http://www.arb-silva.de/>), and added to the ARB database (Ludwig *et al.*, 2004). Putative chimeric sequences were identified with the Mallard program (Ashelford *et al.*, 2006) after the sequences were aligned with that of *Escherichia coli* (U00096) using CLUSTAL_X (Thompson *et al.*, 1997). Forty-seven reference sequences of the phylogenetically related bacteria and environmental clones were selected as authentic sequences located at the periphery of the class *Chlorobea*. After alignment using the ARB program (Ludwig *et al.*, 2004), phylogenetic trees were constructed by the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) methods by using CLUSTAL_X (Saitou & Nei, 1987; Thompson *et al.*, 1997), MORPHY (Adachi & Hasegawa, 1995; Hasegawa *et al.*, 1985) and PAUP version 4 (Swofford, 1998), respectively, using the method and parameters described by Mori *et al.* (2003). In addition, the posterior probabilities of branching points were estimated by Bayesian inference using MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), using the method and parameters described by Mori *et al.* (2008). In the phylogenetic trees constructed using the NJ and ML methods, strain Mat9-16^T formed a phylogenetic lineage with environmental clone sequences around the lineage of green sulfur bacteria. This lineage consisted of some sublineages in the phylogenetic tree that comprised sequences belonging to the green sulfur bacteria and the phylum *Bacteroidetes* (NJ, ML, MP and Bayesian) (Fig. 2). Strain Mat9-16^T was placed into the candidate lineage ZB1 reported by Elshahed *et al.* (2003), and it was supported with a high bootstrap value (99–100%) by the four phylogenetic analysis methods. The maximum 16S rRNA gene sequence divergence for the lineage accommodating the candidate lineage ZB1 was 23.0%.

The presence of the four genes that encode proteins related to photosynthesis, namely *aclB*, *bchG*, *fmoA* and *pscB* genes, was examined in strain Mat9-16^T and *Chlorobaculum tepidum* NBRC 103806^T by PCR amplification using primer sets and the cycling parameters described by Campbell *et al.* (2003), Garcia-Gil *et al.* (2003), Alexander *et al.* (2002) and Figueras *et al.* (2002), respectively. The *aclB* gene encodes

Fig. 2. Neighbour-joining tree of strain Mat9-16^T, related bacteria and environmental clones based on 16S rRNA gene sequences. Open circles at branching nodes indicate supporting probabilities above 95 % by all the phylogenetic analysis methods (NJ, ML, MP and Bayesian), and solid circles indicate probabilities above 85 % by two or more analyses. Bootstrap probabilities are indicated at the corresponding nodes (NJ/ML/MP/Bayesian: A, 49/73/-/-; B, 100/100/99/100; C, 100/100/100/100; D, 94/88/60/97; E, 89/99/61/100; F, 100/100/100/100; G, 99/99/-/100; H, 100/100/99/100). Bar, 0.02 substitutions per nucleotide position.

ATP citrate lyase, which is a key enzyme in the reductive tricarboxylic acid pathway of carbon dioxide fixation during autotrophic growth of prokaryotes, the *bchG* gene encodes an enzyme catalysing the esterification of bacteriochlorophyll (BChl) *a* with geranylgeraniol, the *fmoA* gene encodes the Fenna-Matthews-Olson (FMO) protein as a water-soluble BChl *a* protein which mediates energy transfer between the chlorosomes and the reaction centre, and the *pscB* gene encodes the PscB iron-sulfur protein, which has the binding motif for two 4Fe-4S clusters analogous to the terminal electron acceptors F_A and F_B. However, none of these genes were detected in strain Mat9-16^T whereas all were detected in *Chlorobaculum tepidum* NBRC 103806^T (Fig. 3).

The 16S rRNA gene sequence analysis revealed that strain Mat9-16^T was affiliated with the candidate lineage ZB1

proposed by Elshahed *et al.* (2003). The lineage ZB1 accommodating strain Mat9-16^T was clearly and completely separated from the lineage of green sulfur bacteria representing the nearest cultivated neighbours, and its independence was strongly supported by the probability scores calculated using all phylogenetic analysis methods. The maximum 16S rRNA gene sequence divergence for the lineages clustered with strain Mat9-16^T and related environmental clone sequences was 23.0 %. This divergence is greater than that found in the phylum *Actinobacteria* (18 %) and the green sulfur bacteria (20 %), and similar to that in the phylum *Proteobacteria* (23 %) (Dojka *et al.*, 2000). Furthermore, the 16S rRNA gene sequence of strain Mat9-16^T had similarities of only 77.4 to 82.7 % with those of the green sulfur bacteria affiliated with the class *Chloroidea*. This similarity is lower

Fig. 3. Detection of genes associated with photosynthesis. M, Marker (200 bp DNA ladder); 1, strain Mat9-16^T; 2, *Chlorobaculum tepidum* NBRC 103806^T.

than the 85% similarity that is used as a cut-off for distinguishing new phyla, as suggested by Hugenholtz *et al.* (1998). However, clear supra-group differentiation between the lineage accommodating strain Mat9-16^T and the class *Chlorobea* was not supported by the phylogenetic analysis. Thus, according to the criterion set by Hugenholtz *et al.* (1998) and on the basis of the phylogenetic position, this lineage was considered novel at a higher hierarchical level such as a class-level taxon in the domain *Bacteria*.

The green sulfur bacteria are currently accommodated in the class *Chlorobea* comprising a single order and family (Cavalier-Smith, 2002; <http://www.bacterio.cict.fr/>). In *Bergey's Manual of Systematic Bacteriology*, the green sulfur bacteria were listed as members of the phylum '*Chlorobi*' because they were phylogenetically distinct from the other phyla in the domain *Bacteria* (Garrity & Holt, 2001). Generally, the green sulfur bacteria have been described as follows: 1) they are anoxygenic phototrophic bacteria that grow only under strictly anaerobic conditions; 2) they have chlorosomes as light-harvesting structures that are attached to the cytoplasmic membranes carrying the photosynthetic reaction centre; 3) the cell appearance of the representative species are either green (grass green) or brown (chocolate brown). However, strain Mat9-16^T could not grow by photoassimilation of organic compounds and only grew fermentatively under strictly anaerobic conditions. Moreover, the cells of this strain were colourless and did not contain chlorosomes for photosynthesis in the cytoplasmic membrane (Table 1). In addition, four genes related to photosynthesis, namely *acfB*, *bchG*, *fmoA* and *pscB* genes, were not detected in strain Mat9-16^T although all the genes were detected in *Chlorobaculum tepidum* NBRC 103806^T. These results show that strain Mat9-16^T does not genetically possess the light-harvesting structures, which are a unique characteristic of the green sulfur bacteria.

In conclusion, strain Mat9-16^T significantly differed from members of any of the genera of green sulfur bacteria on the basis of the above-mentioned phylogenetic position,

Table 1. Taxonomic properties of strain Mat9-16^T and green sulfur bacteria

+, Positive; −, negative.

Characteristic	Strain Mat9-16 ^T	Green sulfur bacteria
Cells	Colourless	Green or brown
Chlorosomes	−	+
Metabolism	Obligate heterotroph	Obligate phototroph

morphology and biochemical and physiological properties. Consequently, a novel species in a new genus, *Ignavibacterium album* gen. nov., sp. nov., is proposed for strain Mat9-16^T.

The candidate lineage ZB1 accommodating the sole representative Mat9-16^T should be separated from the green sulfur bacteria, namely the class *Chlorobea*, and the novel class *Ignavibacteria* classis nov. be proposed for it. The proposal of the novel class follows the description of the new family and the new order for this organism, *Ignavibacteriaceae* fam. nov. and *Ignavibacteriales* ord. nov., respectively, as described below. In addition, we also formally propose the tentative phylum-level taxon '*Chlorobi*' as *Chlorobi* phyl. nov., based on our study and the proposals of Garrity & Holt (2001) and Hugenholtz (2002).

Description of *Ignavibacterium* gen. nov.

Ignavibacterium (Ig.na.vi.bac.te'ri.um. L. adj. *ignavus* lazy; L. neut. n. *bacterium* rod; N.L. neut. n. *Ignavibacterium* a lazy rod).

Strictly anaerobic, moderately thermophilic, neutrophilic and obligately heterotrophic bacteria. No photosynthetic growth is observed. Gram-staining negative. Non-sporulating and non-motile. Cells form short to long rods. Catalase-negative and oxidase-negative. The G+C content of genomic DNA is 33.5 mol%. The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. The major isoprenoid quinone is MK-7. Represent a distinct phylogenetic lineage in the family *Ignavibacteriaceae*, the order *Ignavibacteriales* and the class *Ignavibacteria* of the phylum *Chlorobi* based on 16S rRNA gene sequence analysis. The type species is *Ignavibacterium album*.

Description of *Ignavibacterium album* sp. nov.

Ignavibacterium album (al'bum. L. neut. adj. *album* white).

The following properties are additional to the genus description. Cells are short to long rods, 0.2–0.3 × 2.0–15.5 µm in size. The cell appearance is colourless. Growth occurs between 30 and 55 °C with optimum growth at 45 °C. The pH range for growth is 6.5–8.0 with an optimum pH around 7.0–7.5. Growth occurs below 3% NaCl (w/v), with optimum growth at 1% (w/v). Fermentative growth occurs on D-glucose, D-mannose,

D-fructose, maltose, cellobiose, D-salicin and sodium pyruvate. No growth occurs on L-arabinose, D-ribose, D-xylose, D-galactose, D-sorbose, rhamnose, melibiose, trehalose, lactose, sucrose, raffinose, melezitose, D-sorbitol, D-mannitol, *myo*-inositol, formate, acetate, propionate, lactate, *n*-butyrate, fumarate, succinate, malate, citrate, gluconate, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol or 2-butanol. Elemental sulfur (1 %, w/v), sulfate (10 mM), thiosulfate (10 mM), sulfite (1 mM), nitrate (10 mM), nitrite (1 mM), Fe(III) oxide (10 mM), Fe(III) citrate (10 mM), Mn(IV) oxide (10 mM), selenate (1 mM), selenite (1 mM), arsenate (1 mM), arsenite (1 mM), fumarate (10 mM) and oxygen [5 % (v/v) in N₂] are not utilized as alternative electron acceptors. The G + C content of the genomic DNA of the type strain is 33.5 mol%.

The type strain is *Ignavibacterium album* Mat9-16^T (=NBRC 101810^T =DSM 19864^T), which was isolated from the bacterial mats of a hot spring in Yumata, Japan.

Description of *Ignavibacteriaceae* fam. nov.

Ignavibacteriaceae (Ig.na.vi.bac.te.ri.a'ce.ae. N.L. neut. n. *Ignavibacterium* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Ignavibacteriaceae* family of the genus *Ignavibacterium*).

The family *Ignavibacteriaceae* is defined on the basis of a phylogenetic tree reconstructed by phylogenetic analysis of the 16S rRNA gene sequences of a single cultivated representative and of environmental clone sequences retrieved mainly from terrestrial habitats. The type genus is *Ignavibacterium*.

Description of *Ignavibacteriales* ord. nov.

Ignavibacteriales (Ig.na.vi.bac.te.ri.al'es. N.L. neut. n. *Ignavibacterium* type genus of the order; -ales ending to denote an order; N.L. fem. pl. n. *Ignavibacteriales* order of the genus *Ignavibacterium*).

The description is the same as that for the family *Ignavibacteriaceae*. The type genus is *Ignavibacterium*.

Description of *Ignavibacteria* classis nov.

Ignavibacteria (Ig.na.vi.bac.te.ri'a. N.L. fem. pl. n. *Ignavibacteriales* type order of the class; -a ending to denote a class; N.L. neut. pl. n. *Ignavibacteria* the *Ignavibacteriales* class).

The description is the same as that for the family *Ignavibacteriaceae*. The type order is *Ignavibacteriales*.

Description of *Chlorobi* phyl. nov.

Chlorobi (Chlo.ro'bi. N.L. neut. n. *Chlorobium* genus of the phylum; dropping ending to denote a phylum; N.L. fem. pl. n. *Chlorobi* phylum of the genus *Chlorobium*).

The phylum *Chlorobi* is defined on the basis of the phylogenetic tree reconstructed by phylogenetic analysis of 16S rRNA gene sequences. The phylum shares a common root with the phylum *Bacteroidetes*, and comprises two classes, *Chlorobea* and *Ignavibacteria*. Gram-negative bacteria that grow under strictly anaerobic conditions. The type order is *Chlorobiales*.

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