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

Takao Iino,† Koji Mori, Yoshihito Uchino, Tatsunori Nakagawa,‡ Shigeaki Harayama and Ken-ichiro Suzuki

NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8, Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

A moderately thermophilic chemoheterotrophic bacterium, strain Mat9-16T, was isolated from microbial mats developed in hot spring water streams from Yumata, Nagano, Japan. Cells of strain Mat9-16T were strictly anaerobic, Gram-stain-negative, non-sporulating, non-motile and short to long rods (2.0–15.5 μm in length). Strain Mat9-16T 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 Mat9-16T 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 Mat9-16T 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 Mat9-16T, to be named Ignavibacterium album gen. nov., sp. nov. (=NBRC 101810T =DSM 19864T). We also propose to place the cultivated bacterial lineage accommodating the sole representative Mat9-16T 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 microorganisms 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 in the hot water springs (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 hyperthermophilic and acidophilic sulfate-reducing archaean 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.

†Present address: Japan Collection of Micro-organisms, RIKEN BioResource Center, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.
‡Present address: College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510, Japan.

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Mat9-16T is AB478415.
This paper describes the isolation of an anaerobic bacterium, strain Mat9-16<sup>T</sup>, 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 O<sub>2</sub>-absorbing and a CO<sub>2</sub>-generating agent (Anaero-Pack; Mitsubishi Gas Chemical).

The basal medium used, designated S medium, was composed of 0.355 g KCl, 0.14 g KH<sub>2</sub>PO<sub>4</sub>, 0.14 g CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.25 g NH<sub>4</sub>Cl, 4.0 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 3.45 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 6.0 g NaCl, 2.0 mg Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, 2.0 g yeast extract (Becton Dickinson), 2.0 g trypticase peptone (BBL), 5.0 g NaHCO<sub>3</sub> 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 N<sub>2</sub>/CO<sub>2</sub> (4:1, v/v), and 10 ml vitamin solution l<sup>−1</sup> (Wolin et al., 1963) and 10 ml Na<sub>2</sub>S/cystain-HCl solution l<sup>−1</sup> (Iino et al., 2009) were added.

Cells of strain Mat9-16<sup>T</sup> 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<sup>T</sup> revealed a cytoplasmic membrane surrounded by a surface layer (Fig. 1c, d). Strain Mat9-16<sup>T</sup> did not contain chlorosomes, which are the light-harvesting structures of green sulfur bacteria. Cells of strain Mat9-16<sup>T</sup> were colourless and stained Gram-negative by conventional Gram staining.

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<sup>−1</sup> to 10<sup>−10</sup>) 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 O<sub>2</sub>-absorbing and CO<sub>2</sub>-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 N<sub>2</sub>/CO<sub>2</sub> (4:1, v/v). The vials were subsequently sealed with tightly-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<sup>T</sup>, was obtained. After purification, the isolate was maintained in GS medium while flushing with N<sub>2</sub>/CO<sub>2</sub> (4:1, v/v). Chlorobaculum tepidum NBRC 103806<sup>T</sup> used as a reference strain was cultivated in NBRC medium no. 855 (http://www.nbrc.nite.go.jp/).

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Fig. 1. Phase-contrast micrograph (a) and transmission-electron micrographs (b–d) of cells of strain Mat9-16<sup>T</sup>. 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<sup>T</sup> was a strict anaerobe capable of growing under a N<sub>2</sub>/CO<sub>2</sub> (4:1, v/v) atmosphere, but could not grow under microaerobic or aerobic conditions. Strain Mat9-16<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> grew fermentatively on D-glucose, D-fructose, maltose, cellobiose, D-salicin and pyruvate (all at 10 mM). No growth occurred on L-arabinose, D-ribose, D-xylene, D-galactose, D-sorbitose, 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 N<sub>2</sub>] were not utilized as alternative electron acceptors in the presence of yeast extract. Strain Mat9-16<sup>T</sup> was susceptible to chloramphenicol, rifampicin, tetracycline and vancomycin (all at 100 μg ml<sup>–1</sup>), weakly susceptible to bacitracin and gentamicin (both at 100 μg ml<sup>–1</sup>), but resistant to ampicillin, kanamycin and streptomycin (all at 100 μg ml<sup>–1</sup>). 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<sub>15:0</sub> (33.9 %) and iso-C<sub>15:0</sub> (28.7 %), as determined by using the MIDi microbial identification system (Microbial ID; Agilent Technologies) based on the method described by Sasser (1990). C<sub>15:0</sub> (4.0 %), C<sub>16:0</sub> (6.5 %), iso-C<sub>17:0</sub> (7.5 %) and anteiso-C<sub>17:0</sub> (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 Komaga & Suzuki (1987). The genomic DNA G+C content of strain Mat9-16<sup>T</sup> was 33.5 mol%, determined by the HPLC method described by Tamaoka & Komaga (1984).

The 16S rRNA gene of strain Mat9-16<sup>T</sup> was amplified by PCR, and an almost-complete 16S rRNA gene sequence (1461 bases) was determined as described previously (Lino et al., 2007). The 16S rRNA gene sequence of strain Mat9-16<sup>T</sup> 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 slurries 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 Chloroboea, 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<sup>T</sup> (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 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 Chloroboea. 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 (Husonbeck & Ronquist, 2001; Ronquist & Huslenbeck, 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<sup>T</sup> 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 constructed using the NJ and ML methods, strain Mat9-16<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> and Chlorobaculum tepidum NBRC 103806<sup>T</sup> 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...
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) with geranylgeraniol, the fmoA gene encodes the Fenna-Matthews-Olson (FMO) protein as a water-soluble BChl 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 FA and FB. However, none of these genes were detected in strain Mat9-16T whereas all were detected in Chlorobaculum tepidum NBRC 103806T (Fig. 3).

The 16S rRNA gene sequence analysis revealed that strain Mat9-16T was affiliated with the candidate lineage ZB1 proposed by Elshahed et al. (2003). The lineage ZB1 accommodating strain Mat9-16T 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 Mat9-16T 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 Mat9-16T had similarities of only 77.4 to 82.7% with those of the green sulfur bacteria affiliated with the class Chloroebae. This similarity is lower...
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 Mat9-16T 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 is either green (grass green) or brown (chocolate brown). However, strain Mat9-16T 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 aclB, bchG, fmoA and pscB genes, were not detected in strain Mat9-16T although all the genes were detected in Chlorobaculum tepidum NBRC 103806T. These results show that strain Mat9-16T does not genetically possess the light-harvesting structures, which are a unique characteristic of the green sulfur bacteria.

In conclusion, strain Mat9-16T significantly differed from members of any of the genera of green sulfur bacteria on the basis of the above-mentioned phylogenetic position, morphology and biochemical and physiological properties. Consequently, a novel species in a new genus, Ignavigibacterium album gen. nov., sp. nov., is proposed for strain Mat9-16T.

The candidate lineage ZB1 accommodating the sole representative Mat9-16T should be separated from the green sulfur bacteria, namely the class Chlorobea, and the novel class Ignavigibacteria 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, Ignavigibacteriaceae fam. nov. and Ignavigibacteriales 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 Ignavigibacterium gen. nov.**

Ignavigibacterium (Ig.na.vi.bac.te’ri.um. L. adj. ignavus lazy; L. neut. n. bacterium rod; N.L. neut. n. Ignavigibacterium 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-C15:0 and anteiso-C15:0. The major isoprenoid quinone is MK-7. Represent a distinct phylogenetic lineage in the family Ignavigibacteriaceae, the order Ignavigibacteriales and the class Ignavigibacteria of the phylum Chlorobi based on 16S rRNA gene sequence analysis. The type species is Ignavigibacterium album.

**Description of Ignavigibacterium album sp. nov.**

Ignavigibacterium 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-salicylate and sodium pyruvate. No growth occurs on L-arabinose, D-ribose, D-xylene, D-galactose, D-sorbitose, 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* Mat9-16ᵀ (=NBRC 101810ᵀ = DSM 19864ᵀ), which was isolated from the bacterial mats of a hot spring in Yumata, Japan.

**Description of Ignavibacteriaceae fam. nov.**

*Ignavibacteriaceae* (L.na.vि.bac.te.रि.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* (L.na.vि.bac.te.रि.a’les. 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 Ignavibacteriaceae classis nov.**

*Ignavibacteriaceae* (L.na.vि.bac.te.रि.i’a. N.L. fem. pl. n. *Ignavibacteriales* type order of the class; -a ending to denote a class; N.L. neut. pl. n. *Ignavibacteriaceae* 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.रि.bि. 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, *Chlorobiales* and *Ignavibacteriales*. Gram-negative bacteria that grow under strictly anaerobic conditions. The type order is *Chlorobiales*.

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**References**


Garrity, G. M. & Holt, J. G. (2001). The type strain is *Ignavibacterium album* Mat9-16ᵀ (=NBRC 101810ᵀ = DSM 19864ᵀ), which was isolated from the bacterial mats of a hot spring in Yumata, Japan.

**Description of Ignavibacteriaceae fam. nov.**

*Ignavibacteriaceae* (L.na.vि.bac.te.रि.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* (L.na.vि.bac.te.रि.a’les. 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 Ignavibacteriaceae classis nov.**

*Ignavibacteriaceae* (L.na.vि.bac.te.रि.i’a. N.L. fem. pl. n. *Ignavibacteriales* type order of the class; -a ending to denote a class; N.L. neut. pl. n. *Ignavibacteriaceae* 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.रि.bि. 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, *Chlorobiales* and *Ignavibacteriales*. Gram-negative bacteria that grow under strictly anaerobic conditions. The type order is *Chlorobiales*.