Chenggangzhangella methanolivorans gen. nov., sp. nov., a member of the family Methylocystaceae, transfer of Methylopila helvetica Doronina et al. 2000 to Albibacter helveticus comb. nov. and emended description of the genus Albibacter

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A Gram-stain-negative, rod-shaped, non-motile and aerobic bacterial strain, designated CHL1T, was isolated from a sludge sample collected from a sewage treatment tank of an agricultural chemical factory. The strain grew at salinities of 0.5–5 % (w/v) NaCl (optimum 2.5 %). Growth occurred at pH 6.0–8.0 (optimum pH 7.0) and 5–40 °C (optimum 28–30 °C). The genomic DNA G+C content was determined to be 70.4 mol%. Q-10 was detected as the respiratory quinone. The major fatty acids (>10 %) were C18:1ω7c and/or C18:1ω6c and C16:0. The polar lipids consisted of diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, two unidentified phospholipids and two unidentified aminophospholipids. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain CHL1T formed a distinct clade with Albibacter methylovorans DSM 22840T and Methylopila helvetica DM9T within the family Methylocystaceae. On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics, the strain merits recognition as a representative of a novel species of a new genus within the family Methylocystaceae, for which the name Chenggangzhangella methanolivorans gen. nov., sp. nov. is proposed. The type strain of the type species is CHL1T (=KCTC 42661T=CCTCC AB 2015175T). In addition, the species Methylopila helvetica Doronina et al. (2000) is proposed to be transferred to the genus Albibacter as Albibacter helveticus comb. nov. (type strain DM9T=CIP 106788=VKM B-2189) on the basis of the phylogenetic analysis. An emended description of the genus Albibacter is also provided.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CHL1T is KF726142.

One supplementary table and four supplementary figures are available with the online Supplementary Material.
The family Methylocystaceae belongs to the order Rhizobiales, within the class Alphaproteobacteria, and at the time of writing, comprises seven genera: Alibibacter, Hansschlegella, Methylocystis, Methylophilus, Methylosinus, Pleomorphomonas, and Terasakiella (Webb et al., 2014; Parte, 2015). Isolation of strains belonging to the family Methylocystaceae was reported from various ecosystems including soil, water, and phyllosphere and rhizosphere of plants (Bowman et al., 1993; Doronina et al., 1998, 2000, 2001; Ivanova et al., 2007; Zou et al., 2013). Members of this family are known to assimilate C\(_{18}\) fatty compounds and are characterized as Gram-staining-negative with the G+C content ranging from 50.7–70.4 mol% (Webb et al., 2014). This paper describes another novel taxon of the family Methylocystaceae isolated from a sludge sample.

Strain CHL1\(^T\) was isolated from a sludge sample collected from a sewage treatment tank at an agricultural chemical factory located in Jiangsu Province, China. Enrichment and serial dilution plating were employed as described by Yang et al. (2014) for isolation and purification of the culture. Isolation was done using the synthetic medium, phosphate-basal minimal (PBM; Kim et al., 2003) medium supplemented with 0.12 mM chlorimuron-ethyl, 1 ml filter-sterilized vitamin solution and 1 ml sterile calcium-magnesium solution. Strain CHL1\(^T\) was preserved in 25% (v/v) glycerol solution at –80°C.

Colonial properties of the isolate were observed after incubation for 3 days at 30°C on PBM agar plates containing 1% methanol (v/v) (PBMM). Cells morphology was examined by light microscopy and scanning electron microscopy (FEI). Gram staining was determined by standard Gram’s reaction, and confirmed by the KOH lysis test as described by Cerny (1978). The temperature range for growth was tested at 4, 10, 20, 25, 30, 37 and 42°C on PBMM agar media, while the pH range for growth was tested in PBMM broth adjusted to pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0.

Table 1. Differential characteristics of strain CHL1\(^T\) from Alibibacter methyllovorans DSM 22840\(^T\), Methylophilus helvetica DSM 22800\(^T\), Hansschlegella zhihuaiae S 113\(^T\) and Hansschlegella plantiphila S1\(^T\).

<table>
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<th>Characteristic</th>
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<tr>
<td><strong>Cell morphology</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Short rod</td>
<td>60.0–80.0 (7.0)</td>
<td>60.0–90.0 (8.0–9.0)</td>
<td>60.0–80.0 (7.0)</td>
<td>Coccoid</td>
<td>Short rod</td>
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<td><strong>pH range for growth</strong></td>
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<td>6.0–8.0</td>
<td>6.0–9.0</td>
<td>6.0–8.0</td>
<td>Coccoid</td>
<td>5.0–9.0</td>
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<td><strong>Optimum NaCl for growth (%)</strong></td>
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<td>3</td>
<td>1.5</td>
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<tr>
<td><strong>Fermentation of glucose</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td><strong>Hydrolysis of:</strong></td>
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<tr>
<td>Tween 60</td>
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<td>–</td>
<td>–</td>
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<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Urease</td>
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<td>N-Acetylglucosamine</td>
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<tr>
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<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<td><strong>Major fatty acids</strong></td>
<td>C(<em>{18}:1)ω7c and/or C(</em>{18}:1)ω6c, C(_{18}:0)</td>
<td>C(<em>{18}:1)ω7c and/or C(</em>{18}:1)ω6c, C(_{18}:0)</td>
<td>C(<em>{18}:1)ω7c and/or C(</em>{18}:1)ω6c, C(_{18}:0)</td>
<td>C(<em>{18}:1)ω7c, C(</em>{16}:0)</td>
<td>C(<em>{18}:1)ω7c, C(</em>{18}:0)</td>
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<tr>
<td><strong>Polar lipids</strong></td>
<td>DPG, PL, PE, PC, APL</td>
<td>DPG, PL, PE, PC, APL, UL</td>
<td>DPG, PL, PE, PC, APL, AL</td>
<td>DPG, PL, PE, PC, APL, AL</td>
<td>DPG, PC, PE, PME, PG</td>
</tr>
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<td><strong>DNA G+C content (mol%)</strong></td>
<td>70.4</td>
<td>66.7</td>
<td>67.1</td>
<td>65.7</td>
<td>68.5</td>
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using the buffer system described by Xu et al. (2005). PBMM plates containing 0, 1, 2, 3, 4, 5 and 6 % (w/v) NaCl were used for NaCl tolerance experiments. Catalase activity was measured by bubble production on addition of a drop of 3 % (v/v) H₂O₂. Oxidase activity was determined by oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine. Biochemical characteristics including hydrolysis of different substrates (cellulose, gelatin, starch and Tweens 20, 40, 60 and 80), milk coagulation and peptonization, nitrate reduction, utilization of urea and production of H₂S were performed following the methods of Gonzalez et al. (1978). The API 20 NE and API ZYM kits (bioMérieux) and the Biolog GEN III microplate system were employed for observing remaining physiological and biochemical characteristics following the manufacturers’ instructions.

Strain CHL1ᵀ was observed to be Gram-stain-negative, aerobic and non-motile. Scanning electron microscopy revealed a short rod shape with size ranging between 0.9–1.0×1.2–1.8 µm (Fig. S1, available in the online Supplementary Material). The strain did not form flagella, endospores or prosthecate. Colonies were white, raised, and circular with smooth edges on PBMM agar, and measured 1.0–2.0 mm in diameter after cultivation for 3 days at 30 °C. Detailed morphological, cultural, physiological and biochemical characteristics of strain CHL1ᵀ are given in the species description. Distinctive phenotypic features of strain CHL1ᵀ in comparison with its phylogenetically closest neighbours are shown in Table 1.

Cells of strain CHL1ᵀ and reference type strains for chemotaxonomic experiments were harvested from PBMM plates after incubation for 3 days at 30 °C (logarithmic growth phase). Cellular fatty acids were derivatized to methyl esters (Sasser, 1990) and analysed by GC (7890GC; Agilent) using the Sherlock Microbial Identification System (Sherlock version 6.1; MIDI) according to the manufacturer’s instructions. Polar lipids were extracted and identified as described by Minnikin et al. (1979). The quinones were extracted according to the method of Collins et al. (1977) and separated by HPLC (Kroppenstedt, 1982). The G+C content of the genomic DNA was calculated by using the HPLC method of Mesomeh et al. (1989).

The major fatty acids of strain CHL1ᵀ were determined to be C₁₈:₁ω₇c and/or C₁₈:ω₆c (75.8 %) and C₁₆:₀ (15.8 %). The polar lipids of strain CHL1ᵀ consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylycholine, two unidentified phospholipids and two unidentified aminophospholipids (Fig. S2). Q-10 (97.7 %) was the major respiratory quinone detected, along with a small amount of Q-9 (2.3 %). The G+C content of the genomic DNA of strain CHL1ᵀ was determined to be 70.4 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). The 16S rRNA gene sequence was aligned manually with reference sequences retrieved from the GenBank database via the BLAST program and the EzTaxon-e server (Kim et al., 2012). The software package MEGA version 5.0 (Tamura et al., 2011) was employed to reconstruct the phylogenetic trees according to neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms. The evolutionary distance matrices of the neighbour-joining and maximum-likelihood methods were calculated by using Kimura’s two-parameter model (Kimura, 1980). Bootstrap analysis was used to evaluate the topology of phylogenetic trees with 1000 replicates (Felsenstein, 1985). On the basis of the 16S rRNA gene sequence similarities and phylogenetic analysis of strain CHL1ᵀ, DNA–DNA relatedness values with closely related strains were calculated using fluorometric microwell-based DNA–DNA hybridization methods (Ezaki et al., 1989; Christensen et al., 2000).

BLAST analysis of the almost-complete 16S rRNA gene sequence of strain CHL1ᵀ (GenBank accession number KF726142) indicated that strain CHL1ᵀ was related to members of the family Methylocystaceae in the class α-Proteobacteria and exhibited highest 16S rRNA gene sequence similarity to Allobacter methylovorans DSM 22840ᵀ (98.1 % 16S rRNA gene sequence similarity), Hansschlegelia zhihuiae S 113ᵀ (97.8 %) and Myethylipila oligotrophs 2395Aᵀ (97.6 %), and less than 97 % with the other members. A total of 17 nearly full-length 16S rRNA gene sequences of closely related type strains representing all genera in the family Methylocystaceae were retrieved for alignment and phylogenetic analysis with strain CHL1ᵀ. The neighbour-joining phylogenetic tree (Fig. 1) showed that strain CHL1ᵀ formed a distinct clade with A. methylovorans DSM 22840ᵀ and Myethylipila helvetica DM9ᵀ (98.1 and 96.0 % 16S rRNA gene sequence similarities, respectively). This phylogenetic relationship was also supported in the trees generated with maximum-likelihood and maximum-parsimony algorithms (Figs S3 and S4). Based on the similarity indices and the phylogenetic analyses, the strains A. methylovorans DSM 22840ᵀ, M. helvetica DSM 22800ᵀ, H. zhihuiae S 113ᵀ and H. plantiphila S1ᵀ were selected as reference strains for comparative studies, while DNA–DNA hybridization experiments were conducted only with A. methylovorans DSM 22840ᵀ and M. helvetica DSM 22800ᵀ. Genomic DNA–DNA relatedness values between strain CHL1ᵀ and the reference strains A. methylovorans DSM 22840ᵀ and M. helvetica DSM 22800ᵀ were determined to be 25±4 % and 22±2 %, respectively (Table S1).

Besides the phylogenetic differences, strain CHL1ᵀ could be differentiated from A. methylovorans DSM 22840ᵀ, M. helvetica DSM 22800ᵀ, H. zhihuiae S 113ᵀ and H. plantiphila S1ᵀ by the characteristics summarized in Table 1. Based on the differentiating characteristics, strain CHL1ᵀ represents a novel species of a new genus in the family Methylocystaceae, for which the name Chenggangzhangella methanolivorans gen. nov., sp. nov. is proposed.

In addition, comparative analysis of M. helvetica DM9ᵀ indicated that the 16S rRNA gene sequence of strain DM9ᵀ (GenBank accession number AF227126) showed 97.2 % sequence similarity with A. methylovorans DSM 22840ᵀ and less than
96% sequence similarity with other known members of the genus *Methylophilia*. The strain was found to form a distinct clade with *A. methylovorans* DSM 22840^T^, separated distantly from other members of the genus *Methylophilia* in all three phylogenetic trees (Figs 1, S3 and S4). It was therefore considered that strain DM9^T^ has been wrongly placed in the nomenclature and should be reclassified as a separate taxon within the genus *Albibacter* for which the name *Albibacter helveticus* comb. nov. is proposed. An emended description of the genus *Albibacter* is also provided.

**Description of Chenggangzhangella gen. nov.**

*Chenggangzhangella* (cheng.gang.zhang.el'la. N.L. fem. dim. n. *Chenggangzhangella* named after a pioneering Chinese microbiologist, in recognition for his work on microbial resources and their utilization).

Cells are Gram-stain-negative, aerobic, non-motile, short rods. Catalase- and oxidase-positive. The major fatty acids are C_{18:1ω7c} and/or C_{18:1ω6c} and C_{16:0}. The polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, two unidentified phospholipids and two unidentified aminophospholipids. The predominant respiratory quinone is Q-10. The G+C content of the genomic DNA of the type strain of the type species is 70.4 mol%. Phylogenetically, the genus is affiliated to the family *Methylocystaceae*.

The type species is *Chenggangzhangella methanolivorans*.

**Description of Chenggangzhangella methanolivorans sp. nov.**


Displays the following characteristics in addition to those given in the genus description. Cells are 0.9–1.0×1.2–1.8 μm. Does not form flagella, endospores or prosthecae. Colonies are white, raised, and circular with smooth edges on PBMM agar and measure 1.0–3.0 mm in diameter after cultivation for 3 days at 30°C. Growth occurs at 5–40°C (optimum 28–30°C). The pH range for growth is pH 6–8 (optimum pH 7.0). Growth occurs in the presence of 0.5–5% (w/v) NaCl (optimum 2.5%), but not in the presence of 6% (w/v) NaCl. Positive for oxidase, catalase, alkaline phosphatase, esterase, esterase lipase, lipase, leucine

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**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain CHL1\(^T\) and type strains of species of related genera in the family *Methylocystaceae*. *Escherichia coli*. ATCC 11775\(^\text{J}^\text{MST01000030}\) was used as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) ≥50% are shown at branch points. Asterisks denote nodes that were also recovered using the maximum-parsimony and maximum-likelihood methods. Bar, 0.02 substitutions per nucleotide position.
arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for urease, amylase, cellulose, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase. Hydrolyses Tween 60, but not Tween 20 or 40. Negative results in milk coagulation, milk peptonization and H₂S production tests. myo-Inositol, L-aspartic acid, L-glutamic acid, D-gluconic acid, glucuronamide, D-saccharic acid, methyl pyruvate, D-malic acid, L-malic acid, bromosuccinic acid, acetic acid and formic acid can be utilized as sole carbon sources, but not dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, D-raffinose, α-D-lactose, melibiase, α-D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, glycerol, gelatin, pectin, L-lactic ccid, citric ccid, α-aminobutyric acid, β-hydroxybutyric acid, α-ketobutyric acid, acetoacetic acid or propionic acid. Positive for acid production from D-glucose, assimilation of L-arabinose, maltose, capric acid, adipic acid and malic acid, but negative for nitrate reduction, indole production, activities of arginine dihydrolase, galactosidase and urease, hydrolysis of aesculin and gelatin, assimilation of D-glucose, D-mannose, D-mannitol, N-acetylgalactosamine, potassium gluconate, trisodium citrate and phenylacetic acid.

The type strain is CHL1<sup>T</sup> (=KCTC 42661<sup>T</sup>=CCTCC AB 2015175<sup>T</sup>) was isolated from a sludge sample collected from a sewage treatment tank at an agricultural chemical factory in Jiangsu Province, China. The genomic DNA G+C content of the type strain is 70.4 mol%.

**Emended description of the genus**

**Albibacter** Doronina et al. 2001

The genus is as described by Doronina et al. (2001) with the following amendments. Cells are motile by means of single lateral flagellum or are non-motile.

**Description of Albibacter helveticus** comb. nov.

*Albibacter helveticus* (hel.ve’ticus L. masc. adj. helveticus from Helvetia, an old name of Switzerland).

Basonym: *Methyloplila helvetica* Doronina et al.

The description of the species is as originally described by Doronina et al. (2000).

The type strain DM9<sup>T</sup> (=CIP 106788<sup>T</sup>=VKM B-2189<sup>T</sup>) was isolated from groundwater in Switzerland as a dichloromethane-utilizing bacterium.

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