**Albibacter methylovorans** gen. nov., sp. nov., a novel aerobic, facultatively autotrophic and methylotrophic bacterium that utilizes dichloromethane

Nina V. Doronina,1 Yuri A. Trotsenko,1 Tatjana P. Tourova,2 Boris B. Kuznetsov3 and Thomas Leisinger4

Author for correspondence: Yuri A. Trotsenko. Tel: +7 095 925 7448. Fax: +7 095 956 3370. e-mail: trotsenko@ibpm.serpukhov.su

A novel genus, *Albibacter*, with one species, *Albibacter methylovorans* sp. nov., is proposed for a facultatively chemolithotrophic and methylotrophic bacterium (strain DM10T) with the ribulose bisphosphate (RuBP) pathway of C\textsubscript{1} assimilation. The bacterium is a Gram-negative, aerobic, asporogenous, non-motile, colourless rod that multiplies by binary fission. The organism utilizes dichloromethane, methanol, methylamine, formate and CO\textsubscript{2}/H\textsubscript{2}, as well as a variety of polycarbon compounds, as carbon and energy sources. It is neutrophilic and mesophilic. The major cellular fatty acids are straight-chain unsaturated C\textsubscript{18:1}, saturated C\textsubscript{16:0} and cyclopropane C\textsubscript{19:0} acids. The main ubiquinone is Q-10. The dominant phospholipids are phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline and cardiolipin. The DNA G+C content is 66.7 mol\%. Strain DM10T has a very low degree of DNA–DNA hybridization (4–7%) with the type species of the genera *Paracoccus*, *Xanthobacter*, *Blastobacter*, *Angulomicrobium*, *Ancylobacter* and *Ralstonia* of RuBP pathway methylobacteria. Another approach, involving comparative 16S rDNA analysis, has shown that the novel isolate represents a separate branch within the \(\alpha\)-2 subgroup of the *Proteobacteria*. The type species of the new genus is *Albibacter methylovorans* sp. nov.; the type strain is DM10\(^\text{T}\) (\(=\) VKM B-2236\(^\text{T}\) = DSM 13819\(^\text{T}\)).

**Keywords:** *Albibacter*, ribulose bisphosphate pathway, methylotroph

INTRODUCTION

Dichloromethane (DCM), or methylene chloride, is a widely used industrial solvent that escapes at a considerable rate into the environment. Both aerobic methylotrophic bacteria (Leisinger *et al.*, 1994) and a strictly anaerobic acetogenic bacterium (Mägli *et al.*, 1998) growing with DCM as the sole carbon and energy source have been isolated. A set of 14 aerobic DCM-utilizing bacteria (strains DM1–DM14) has previously been isolated from soils and waters contaminated with the compound (Brunner *et al.*, 1980; Stucki *et al.*, 1981; Gaelli & Leisinger, 1985; Scholtz *et al.*, 1988). In all of these strains, the initial step of DCM utilization is catalysed by a glutathione-dependent DCM dehalogenase. This enzyme transforms DCM plus one molecule of water to formaldehyde and two molecules of hydrochloric acid. The overall reaction is thought to involve the enzyme-catalysed dehalogenative formation of an S-chloromethyl glutathione conjugate, which subsequently undergoes hydrolysis to formaldehyde, glutathione and hydrochloric acid (Leisinger *et al.*, 1994). Extensive phenotypic and genotypic characterization of the 14 aerobic DCM utilizers has led to the classification of seven representatives (strains DM1, DM3 and DM5–DM9\(^\text{T}\)) as *Methylophilus leisingeri* (Doronina *et al.*, 2000). Four further representatives were classified as *Methylobacterium dichloromethanicum* DM4\(^\text{T}\) (Doronina *et al.*, 2000), *Methylorhabdus multivorans* DM13\(^\text{T}\) (Doronina *et al.*, 1995), *Methylophilus leisingeri* DM11\(^\text{T}\).
(Doronina & Trotsenko, 1994) and *Paracoccus methylovorans* DM12<sup>5</sup> (Doronina et al., 1998a). Like the latter organism, one of the remaining unidentified isolates (strain DM10<sup>5</sup>) had the ribulose bisphosphate (RuBP) pathway for C<sub>1</sub> assimilation (Doronina et al., 1992), but differed from known facultatively autotrophic methylotrophic bacteria in some other properties.

The group of aerobic Gram-negative, asporogenous, facultatively autotrophic and methylotrophic bacteria having the RuBP pathway and capable of growth on a variety of reduced C<sub>1</sub> substrates, except methane, is very heterogeneous and includes the genera *Paracoccus*, *Xanthobacter*, *Blastobacter*, *Anguimicrobium*, *Ralstonia* and *Ancylobacter*. The representatives of *Xanthobacter* form pleomorphic cells, which are slimy and yellow due to the water-insoluble carotenoid pigment zeaxanthin dirhamnozide (Wiegel, 1992; Urakami et al., 1995). Species of the genera *Blastobacter* (irregular ovoid pleomorphic rods) (Sly, 1985; Trotsenko et al., 1989) and *Anguimicrobium* (tetrahedron-shaped cells) (Vasilyeva et al., 1979; Vasilyeva, 1989) multiply by budding. The vibrioid cells of the genus *Ancylobacter* (Raj, 1981, 1983) have a characteristic morphology and rings that are formed occasionally prior to cell division. *Ralstonia* (formerly *Alcaligenes*) species are motile by one to eight peritrichous flagella (Kersters & De Ley, 1984). This report presents the results of our polyphasic taxonomy studies, indicating that strain DM10<sup>5</sup> should be placed in a novel genus, for which the name *Albibacter* gen. nov. is proposed; the genus has a single species, *Albibacter methylovorans* sp. nov.

### METHODS

**Bacterial strains.** Strain DM10<sup>5</sup> was isolated on DCM agar from a ground-water sample by a procedure described previously (Gaelli & Leisinger, 1985). The organisms used for comparative studies included the following reference strains of aerobic, facultatively autotrophic and methylotrophic bacteria: *Paracoccus denitrificans* ATCC 17441<sup>5</sup>, *Paracoccus methylutens* VKM B-2164<sup>5</sup>, *Paracoccus aminovorans* JCM 7685<sup>6</sup>, *Paracoccus aminovorans* JCM 7686<sup>6</sup>, *Xanthobacter autotrophicus* DSM 432<sup>6</sup>, *Blastobacter denitrificans* IFAM 1005<sup>5</sup>, *Anguimicrobium tetradraele* Z-2821<sup>7</sup> (= VKM B-1335<sup>5</sup>), *Ancylobacter aquaticus* ATCC 25396<sup>6</sup>, *Ralstonia eutropha* DSM 428 and *Methylophilus capsulatus* VKM B-1606<sup>6</sup>. They were maintained on PYG agar medium, which contained 0.5% peptone, 0.5% yeast extract, 0.5% glucose and 2% Difco Bacto agar (pH 7.0). Strain DM10<sup>5</sup> was grown on minimal medium K, which contained (g l<sup>−1</sup>): KH<sub>2</sub>P<sub>2</sub>O<sub>4</sub> 2.0; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.025; NaCl 0.5. Prior to autoclaving, the pH of the medium was adjusted to 7.2. After sterilization, an appropriate carbon source and 0.02% (v/v) yeast autolysate were added and 1l medium was supplemented with 1 ml trace element solution (Doronina et al., 1995). Methanol or ethylamine was added at concentrations of 0.5 or 0.3% (v/v), respectively. Unless otherwise mentioned, the bacteria were grown at 29 °C in 750 ml Erlenmeyer flasks containing 200 ml medium on a rotary shaker at 180 r.p.m. During cultivation with DCM, the flasks were closed tightly with rubber stoppers. Since the pH of the medium decreased when DCM was consumed, the compound was added in three portions to a final concentration of 10 mM (0.65 g l<sup>−1</sup>), each after the pH of the medium had been adjusted to 7.2 with NaOH. Other sterile carbon sources were added from filter-sterilized stock solutions. The concentrations of the tested carbon sources ranged from 0.5 and 3.0 g l<sup>−1</sup>. For growth on agar-solidified medium with DCM, Petri dishes were incubated in a desiccator, 0.1 ml portions of DCM being added to a reagent tube three times, each after 48 h. The bacterial strains were maintained on liquid minimal medium K for 10 d on agar-solidified slants with an appropriate C<sub>1</sub> substrate at 4 °C for 2 weeks and in the freeze-dried state with a protectant (skimmed milk) for over a year.

**Identification methods.** If not otherwise indicated, the methods, reagents and media used for phenotypic characterization were as described by Smibert & Krieg (1994). Cell morphology, Gram staining, motility and flagellation were determined by using cultures grown on PYG agar. Production of a fluorescent pigment was tested on King A and B agar-solidified media. Nitrate reduction was tested in liquid medium K, in which (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was replaced by 0.5% (w/v) KNO<sub>3</sub>, after 1.5 and 5 d incubation. The methyl red and Voges–Proskauer (acetoin) reactions were tested in glucose/phosphate/peptone broth. Indole production was determined by the Salkowski reagent (Gordon & Weber, 1951) in medium K with 0.5% methanol and 1% tryptophan. Hydrogen sulfide production was tested on triple-sugar-iron agar for 2 weeks. Gelatin hydrolysis was observed in stab cultures in yeast extract/peptone medium containing 12% gelatin after 4 weeks incubation at 22 °C. Starch hydrolysis was determined by using an iodine solution on agar-solidified medium K containing 0.2% soluble starch after 1.5 and 10 d incubation.

Ammonia production was tested in peptone water by using Nessler’s reagent. The oxidase activity test was performed with a 1% solution of tetramethyl-p-phenylenediamine dihydrochloride. Urease activity was observed on Christensen’s medium. Catalase activity was detected by pouring a 3% H<sub>2</sub>O<sub>2</sub> solution into colonies on solid medium K or PYG agar. Halotolerance was tested by inoculating the cells into liquid medium K with various concentrations of NaCl (1–14%). Growth at different temperatures and pH values was tested in liquid medium K with 0.5% (v/v) methanol. Utilization of a wide range of growth substrates (> 50) was also determined in liquid medium K after 2 weeks cultivation, with methanol being replaced by the other carbon compounds. Organic acids and amino acids were added at concentrations of 0.05–0.30%, whereas carbohydrates and alcohols were added at concentrations of 0.2–0.5%.

Methane utilization was tested in an atmosphere containing methene and air (1:1, v/v) in 700 ml conical flasks containing 100 ml medium K and fitted with rubber stoppers. Hydrogen utilization was tested by the same procedure, but in an atmosphere of H<sub>2</sub>/O<sub>2</sub>/CO<sub>2</sub> (7:2:1, by vol.). Utilization of chloromethane was tested as described for DCM (Doronina et al., 1995). To test alternative nitrogen sources, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was replaced by other nitrogen compounds.

Enzyme assays were done as described previously (Trotsenko et al., 1986; Doronina et al., 1995). Cellular phospholipids, ubiquinones and fatty acids were analysed according to methods described previously (Doronina et al., 1998a, b).

**Electron-microscope analysis.** A cell suspension was mounted
on a Formvar-coated copper grid and stained with 0.2% (w/v) phosphotungstic acid (pH 7.2). For thin sectioning, samples were prefixed with 1.5% (v/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) and washed three times with 1% (w/v) OsO4 in 0.05 M cacodylate buffer (pH 7.2) for 3 h at 20°C. After dehydration in an alcohol series, the cells were embedded in Spurr epoxy resin and sectioned with an LKB 2128 Ultratome. The ultrathin sections were mounted on copper grids and double-stained with uranyl acetate and lead citrate. Negatively stained preparations and thin sections were imaged in a JEOL JEM-100B transmission electron microscope at an operating voltage of 60 kV.

**DNA isolation and characterization.** DNA was isolated and purified according to Marmur (1961). Its G+C content was determined by the thermal denaturation (Tm) method with a Beckman DU-8B spectrophotometer at a heating rate of 0.5°C min-1 and calculated according to Owen & Lapage (1976). DNA from *Escherichia coli* K-12 was used as a standard. DNA–DNA hybridizations were done on nitrocellulose membrane filters (0.22 μm) according to the procedure of Denhardt (1966) in the following incubation mixture: 0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0, with 30% formamide at 60°C for 24 h. Deoxy[1,2,5-3H]CTP and the nick translation kit N 5500 (Amersham) were used to label the DNA probes.

**16S rDNA amplification and sequencing.** Genomic DNA was isolated by standard phenol/chloroform extraction. The 16S rRNA gene was selectively amplified by PCR using the following primers: forward primer, 5′-AGAGTTTGTATCCTGGCTCAG-3′ (8–27 by *E. coli* numbering); reverse primer, 5′-TACGGTACCTTGTTACGACTT-3′ (1471–1492 by *E. coli* numbering) (Lane, 1991). The PCR was carried out in a final volume of 100 μl, with 1 μg DNA template, 200 μM each primer, 200 μM each dNTP, 3 U Tetratex polymerase (BioMaster) and reaction buffer (100 mM Tris/HCl, pH 8.3, 500 mM KCl, 2 mM MgCl2). The temperature cycling was done by using 30 cycles of the following thermal profile: 1 min at 94°C, 1 min at 42°C and 1 min at 72°C. The final extension was carried out at 72°C for 6 min. The PCR products were purified using the PCR-prep kit (Promega) according to the manufacturer’s instructions. DNA sequencing was carried out by using the Sequenase version 2 kit (USB) with minor modifications.

**Phylogenetic analysis.** The 16S rDNA sequence of strain DM10T was manually aligned to representative protobacterial sequences obtained from the GenBank database. Regions that were not sequenced in one or more reference organisms were omitted from the final analysis and 1236 nt in total were used for tree construction. Pairwise evolutionary distances (expressed as estimated changes per nucleotide) were used for tree construction. Pairwise evolutionary distances (expressed as estimated changes per nucleotide) were used for tree construction.

**Results**

**Morphology**

Strain DM10T was a Gram-negative, non-motile short rod, 0.9–1.0 × 1.2–1.8 μm in size, with a cell shape that sometimes approached coccoid (Fig. 1a, b). It did not form flagella, endospores or prosthecae. Cells occurred singly or in pairs and sometimes in clusters. Reproduction was by binary fission. Special subcellular structures related to the autotrophic or methylotrophic modes of life, such as carboxysomes or intracytoplasmic membranes, were not observed.

**Phenotypic characteristics**

Colonies on PYG agar (3 d) were punctate, round, convex, lustrous, white, transparent, uniform in consistency and mucoid. Streak cultures on meat/peptone agar were distinct and white, with even edges, a smooth surface and a mucoid consistency. Growth was moderate and odourless. No water-soluble fluorescent pigment was produced. Nitrate was reduced to nitrite. Isolate DM10T hydrolysed starch (weakly), but not gelatin, cellulose or casein milk. It formed indole from tryptophan on medium with nitrate as a nitrogen source. Methyl red and Voges–Proskauer tests were negative. Urease and catalase were present, but oxidase activity was very low.

Strain DM10T grew well on mineral salt liquid medium with a gas mixture of CO2:H2/O2, DCM (optimal concentration: 0.05–0.1%, v/v), methanol (0.5%, v/v), methylamine (0.3%, w/v) or formaldehyde (0.05%, w/v) as the carbon and energy sources. Growth on C1 substrates was stimulated by addition of yeast autolysate (0.0% 1%) or thiamin/biotin (both at 20 μg l-1). Growth was also supported by glucose, fructose, arabinose,

---

**Fig. 1.** Electron micrographs of cells of strain DM10T. Upper micrograph, negatively stained preparation; lower micrograph, ultrathin section showing cell structure. Bars, 1 μm.
Table 1. Cellular fatty acid composition of strain DM10T grown on PYG agar (48 h)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percentage of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iso 15:0</td>
<td>0.3</td>
</tr>
<tr>
<td>Anteiso 15:0</td>
<td>1.5</td>
</tr>
<tr>
<td>Iso 16:0</td>
<td>0.7</td>
</tr>
<tr>
<td>16:1</td>
<td>1.5</td>
</tr>
<tr>
<td>16:0</td>
<td>19.7</td>
</tr>
<tr>
<td>Anteiso 17:0</td>
<td>1.0</td>
</tr>
<tr>
<td>Unsaturated 18</td>
<td>11.6</td>
</tr>
<tr>
<td>c18:1ω7</td>
<td>57.6</td>
</tr>
<tr>
<td>18:0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 2. Enzyme activities in cell extracts of the strain DM10T grown with DCM or methylamine

<table>
<thead>
<tr>
<th>Enzyme (co-substrates)</th>
<th>Enzyme activity [nmol min(^{-1}) (mg protein(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM dehalogenase (GSH)</td>
<td>118</td>
</tr>
<tr>
<td>Methylamine dehydrogenase (PMS)</td>
<td>0</td>
</tr>
<tr>
<td>Formaldehyde dehydrogenase (NAD(^+), GSH)</td>
<td>700</td>
</tr>
<tr>
<td>Formate dehydrogenase (PMS)</td>
<td>50</td>
</tr>
<tr>
<td>Hydroxypyruvate reductase (NADPH)</td>
<td>120</td>
</tr>
<tr>
<td>Phosphoribulokinase</td>
<td>70</td>
</tr>
<tr>
<td>Ribulose-1,5-bisphosphate carboxylase</td>
<td>120</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (NAD(^+))</td>
<td>60</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (NADP(^+))</td>
<td>60</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrogenase (NAD(^+))</td>
<td>64</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrogenase (NADP(^+))</td>
<td>64</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphate aldolase</td>
<td>30</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase (NAD(^+))</td>
<td>17</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>27</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase (NAD(^+))</td>
<td>22</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase (NADP(^+))</td>
<td>362</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase (NAD(^+))</td>
<td>26</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>12</td>
</tr>
<tr>
<td>Malate synthase</td>
<td>16</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (NADPH)</td>
<td>75</td>
</tr>
<tr>
<td>Glutamate synthase (NADPH)</td>
<td>21</td>
</tr>
<tr>
<td>Glutamine synthetase (ADP, Mn(^{2+}))</td>
<td>30</td>
</tr>
</tbody>
</table>

and DMSO did not support growth. Strain DM10\(^T\) utilized methylamine, some amino acids, urea, peptone, ammonium salts and nitrates as nitrogen sources. Good growth occurred between pH 6.0 and 9.0, but not above pH 9.5 or below pH 6.0. The optimum growth temperature was 28–30 °C and the pH optimum was 7.5–8.0. Growth did not occur in the presence of 3% NaCl. The generation time was 0.08 h\(^{-1}\) on medium K with 10 mM DCM. Strain DM10\(^T\) grew as an aerobic facultative chemolithotroph with a respiratory type of metabolism using oxygen as the terminal electron acceptor. No anaerobic growth was observed in the presence of potassium nitrate. The organism was resistant to penicillin and rifampicin, but sensitive to gentamicin, kanamycin, ampicillin, neomycin, novobiocin, nalidixic acid, tetracycline and lincomycin (all at 30 µg ml\(^{-1}\)).

Chemotaxonomic characteristics

As shown in Table 1, the dominant cellular fatty acids of strain DM10\(^T\) were cis-vaccenic (c18:1ω7) and palmitic (16:0) acids. Analysis of the cellular phospho-
Albibacter, novel aerobic methylobacteria

Fig. 2. Unrooted phylogenetic tree showing relationships between Albibacter methyllovorans DM10\textsuperscript{T} and other members of the \(\alpha\)-Proteobacteria, including methylotrophic genera. The 16S rDNA sequence obtained in this study is shown in bold. Bootstrap values (expressed as percentages of 100 replications) are shown at the appropriate branch points. Bar, Jukes & Cantor evolutionary distance.

Metabolic characteristics

In order to establish the routes of primary and intermediary C\(_1\) metabolism in strain DM10\textsuperscript{T}, enzyme activities were determined in extracts of cells grown on DCM or methylamine. As shown in Table 2, DCM-grown cells contained an inducible, reduced glutathione (GSH)-dependent DCM dehalogenase, whereas methylamine-grown cells possessed methylamine dehydrogenase. Formaldehyde was further oxidized by GSH-dependent formaldehyde dehydrogenase to formate. The latter was finally oxidized to CO\(_2\) by phenazine methosulfate (PMS)-linked formate dehydrogenase. Carbon dioxide was assimilated via the RuBP pathway (Benson–Calvin cycle), as confirmed by the presence of phosphoribulokinase and ribulose-1,5-bisphosphate carboxylase. Neither the serine nor the ribulose monophosphate pathway of C\(_1\) assimilation was operative, due to the absence of the appropriate specific enzymes: hydroxypropionate reductase (NADH), serine-glyoxylate aminotransferase, malate lyase and hexulose-phosphate synthase. Strain DM10\textsuperscript{T} had no 2-keto-3-deoxy-6-phosphogluconate aldolase; therefore the Entner–Doudoroff pathway did not function. In addition, the enzymes of the pentose phosphate pathway (glucose-6-phosphate and 6-phosphogluconate dehydrogenases) were present at low levels. Hence, fructose-1,6-bisphosphate aldolase might play an important role in the glycolytic conversion of phosphohexoses and phosphotrioses. Strain DM10\textsuperscript{T} contained pyruvate dehydrogenase as well as a complete set of the tricarboxylic acid cycle and glyoxylate shunt enzymes. Primary assimilation of ammonia occurred by both reductive amination of 2-oxoglutarate and through the glutamate cycle (the GS/GOGAT system).

Genomic characteristics

The G+C content of DNA of strain DM10\textsuperscript{T} was 66.7 mol\% (\(T_m\) method). To estimate the extent of DNA similarity of this strain with DNAs from representatives of other bacterial taxa, the filter hybridization method was used. Strain DM10\textsuperscript{T} showed low levels of DNA–DNA relatedness (2–7\%) to the following strains: \(P.\) denitrificans ATCC 17441\textsuperscript{T}, \(P.\) methyllovorans VKM B-2164\textsuperscript{T}, \(P.\) aminovorans JCM 7686\textsuperscript{T}, \(P.\) aminophilus JCM 7686\textsuperscript{T}, \(Xanthobacter autotrophicus\) DSM 432\textsuperscript{T}, \(Blastobacter denitrificans\) IFAM 1005\textsuperscript{T}, \(Blastobacter capsulatus\) IFAM 1004\textsuperscript{T}, Angulo-
Microbium tetraedrale Z-2821T, Ancylobacter aquaticus ATCC 25396T,Ralstonia eutropha DSM 428 and Methyloptila capsulata VKM B-1606T.

**Comparative 16S rDNA sequence analysis**

A total of 1409 nt of the 16S rDNA sequence was determined for strain DM10T, corresponding to positions 19–1482 of the E. coli sequence. The sequence was compared with the appropriate sequences of some representatives of the α-Proteobacteria, including RuBP-pathway methylotrophs belonging to the genera Paracoccus, Xanthobacter, Blastobacter and Ancylobacter. Surprisingly, the matrix of full sequence similarity values for strain DM10T and representative members of the Proteobacteria indicated a rather high identity (95.4 %) to a recently described member of the α-2 subgroup, Methyloptila capsulata (Doronina et al., 1998b). The degree of sequence similarity to the other members of the α-2 subgroup was significantly lower (86.3–90.5%). The lowest level of similarity (86.0%) was found to P. denitrificans, a member of the α-3 subgroup. As seen in Fig. 2, strain DM10T formed a single cluster with the type strain of the serine-pathway methylotroph Methyloptila capsulata (bootstrap value of 100%). However, the scale of evolutionary changes in the 16S rRNA gene of strain DM10T in comparison with that of the related α-2 subgroup of the Proteobacteria did not correspond to profound (essential) changes in the phenotype of the bacterium. Hence, considering sequence homology as part of a polyphasic taxonomy approach to classification and identification of these methylotrophs, further genotypic and phenotypic information must also be taken into account (Vandamme et al., 1996). In this context, a very low degree of DNA–DNA hybridization (< 5%) and some essential chemotaxonomic and biochemical differences, particularly the operation of the RuBP pathway in strain DM10T, argue strongly against its formal generic clustering with Methyloptila capsulata.

**DISCUSSION**

Six generic names have been validly published for Gram-negative RuBP-pathway methylotrophs: Paracoccus, Xanthobacter, Blastobacter, Angulomicrobium, Ancylobacter and Ralstonia (formerly Alcaligenes). Our isolate differed from the members of these genera in some essential phenotypic and genotypic characteristics. Phylogenetic analysis confirmed the absence of close relatedness between strain DM10T and species of the genera Xanthobacter and Blastobacter. Although strain DM10T resembled Paracoccus species on the basis of morphology, ubiquinone system, pathway of C4 assimilation and range of DNA G + C content, it had very low DNA–DNA similarities (5–7%) to the type species of this genus and thus belongs to another subgroup of the Proteobacteria. Despite rather high 16S rDNA similarity to the type strain of Methyloptila capsulata, strain DM10T was clearly distinct from this serine-pathway methylotroph in some morphological features, cellular phospholipids and fatty acids, autotrophic growth and operation of the RuBP pathway, as well as by virtue of a very low DNA–DNA hybridization value. Based on these results and adopting a polyphasic approach to taxonomy (Vandamme et al., 1996), strain DM10T should be placed in a separate taxon, the genus Albibacter gen. nov. Phylogenetic relationships that were derived from the 16S rDNA sequence analysis showed that the new genus formed a distinct branch within the α-2 subgroup of the Proteobacteria. The genus Albibacter contains a single species, which is automatically the type species of the genus, and it is therefore proposed that the new species be named Albibacter methylvorans sp. nov.

**Description of Albibacter gen. nov.**

Albibacter (Al.bi.bac.ter. L. adj. albus white; M.L. masc. n. bacter equivalent of Gr. neut. n. baktron a small rod; M.L. masc. n. Albibacter white rod).

Cells are Gram-negative, non-sporulating, colourless rods that occur singly, in pairs or in clusters and multiply by binary fission. Colonies on methanol or PYG agar are circular, 1–2 mm in diameter, white, convex, translucent to opaque and mucoid. Methyl red and Voges–Proskauer tests are negative. Does not produce water-soluble fluorescent pigment. Indole is produced from tryptophan on medium with nitrate as nitrogen source. Reduces nitrate to nitrite. No anaerobic growth in the presence of nitrate. Produces acids from sugars oxidatively, but not fermentatively. Hydrolyses starch, but not gelatin or cellulose. Chemo-lithoheterotrophic and facultatively methylotrophic, assimilating C1 compounds by the RuBP pathway. Able to grow on a wide spectrum of polycarbon substrates. Ammonium salts, nitrate, urea, peptone, some amino acids and methylamine are utilized as nitrogen sources. Strictly aerobic, with a respiratory type of metabolism. Neutrophilic and mesophilic. Grows at pH 6.0–9.0 and at temperatures between 10 and 37 °C. No growth in the presence of 3% NaCl. The major ubiquinone is Q-10. Dominant phospholipids are phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline and phosphoserine. The prevailing cellular fatty acids are cis-vaccenic (c18:1ω7) and palmitic (16:0) acids. Levels of DNA–DNA hybridization with representatives of the genera Paracoccus, Xanthobacter, Blastobacter, Angulomicrobium, Ralstonia and Methyloptila are less than 7%. The genus Albibacter belongs to the α-2 subgroup of the Proteobacteria, as revealed by comparative sequence analysis of the 16S rDNA. The type species is Albibacter methylvorans.

**Description of Albibacter methylvorans sp. nov.**

Albibacter methylvorans (me.thy.lo.vor’ans. M.L. n. methylum the methyl group; L. part adj. vorans devouring, digesting; methylvorans digesting methyl groups).
In addition to the characteristics that define the genus, it has the characteristics described below. Cells are non-motile short rods, 0.9–1.0 µm in diameter and 1.2–1.8 µm in length. Urease- and catalase-positive. Oxidase activity is very low. Optimal growth at pH 7.5–8.0 and temperatures of 28–30 °C. Utilizable carbon sources are DCM, methanol, methylamine, formate, CO₂/H₂, d-glucose, d-fructose, D-mannose, L-arabinose, d-xylene, sorbose, ribose, maltose, sucrose, d-sorbitol, D-mannitol, inositol, glycerol, dulcitol, adonitol, ethanol, acetate, 2-oxoglutamate, fumarate, pyruvate, succinate, malate, citrate, oxaloacetate, propionate, cis-aconitate, l-glutamate, l-alanine and acetamide. The following are not utilized: methane, chloromethane, formaldehyde, di- and trimethylamines, thiocyanate, thiosulfate, DMSO and dimethyl acetamide. Yeast extract (0.01%, w/v) or a biotin/thiamin mixture (both at 20 µg l⁻¹) stimulates growth. Resistant to penicillin and rifampicin, but sensitive to gentamicin, kanamycin, ampicillin, neomycin, novo-norbiocin, nalidixic acid, tetracycline and lincomycin. The type strain, DM10, shows resistance to penicillin and rifampicin, but is sensitive to gentamicin, kanamycin, ampicillin, neomycin, novobiocin, nalidixic acid, tetracycline and lincomycin. The DNA G+C content is 66.7 mol% (T₂ method). The type strain, DM10¹ (VKM B-2236⁰ = DSM 13819⁰), was isolated in Switzerland from ground water contaminated with DCM.

ACKNOWLEDGEMENTS

This work was funded by grant INTAS 94-3122.

REFERENCES


