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

¹ Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russia

^{2,3} Institute of Microbiology² and Centre 'Bioengineering'³, Russian Academy of Sciences, Moscow 117811, Russia

⁴ Institut für Mikrobiologie, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zürich, Switzerland

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 DM10^T) with the ribulose biphosphate (RuBP) pathway of C₁ 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 CO₂/H₂, 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_{18:1}, saturated C_{16:0} and cyclopropane C_{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 DM10^T 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 methylotrophs. Another approach, involving comparative 16S rDNA analysis, has shown that the novel isolate represents a separate branch within the α -2 subgroup of the *Proteobacteria*. The type species of the new genus is *Albibacter methylovorans* sp. nov.; the type strain is DM10^T (= VKM B-2236^T = DSM 13819^T).

Keywords: *Albibacter*, ribulose biphosphate 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^T) as *Methylophilus helveticus* (Doronina *et al.*, 2000). Four further representatives were classified as *Methylobacterium dichloromethanicum* DM4^T (Doronina *et al.*, 2000), *Methylobacterium multivorans* DM13^T (Doronina *et al.*, 1995), *Methylobacterium leisingeri* DM11^T

Abbreviations: DCM, dichloromethane; GSH, reduced glutathione; PMS, phenazine methosulfate; RuBP, ribulose biphosphate.

The GenBank accession number for the 16S rDNA sequence of *Albibacter methylovorans* DM10^T is AF273213.

(Doronina & Trotsenko, 1994) and *Paracoccus methylutens* DM12^T (Doronina *et al.*, 1998a). Like the latter organism, one of the remaining unidentified isolates (strain DM10^T) had the ribulose biphosphate (RuBP) pathway for C₁ 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 C₁ substrates, except methane, is very heterogeneous and includes the genera *Paracoccus*, *Xanthobacter*, *Blastobacter*, *Angulomicrobium*, *Ralstonia* and *Ancylobacter*. The representatives of *Xanthobacter* form pleomorphic cells, which are slimy and yellow due to the water-insoluble carotenoid pigment zeaxanthin dirhamnoide (Wiegel, 1992; Urakami *et al.*, 1995). Species of the genera *Blastobacter* (irregular ovoid pleomorphic rods) (Sly, 1985; Trotsenko *et al.*, 1989) and *Angulomicrobium* (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 (Kerstens & De Ley, 1984). This report presents the results of our polyphasic taxonomy studies, indicating that strain DM10^T 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^T 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^T, *Paracoccus methylutens* VKM B-2164^T, *Paracoccus aminovorans* JCM 7685^T, *Paracoccus aminophilus* JCM 7686^T, *Xanthobacter autotrophicus* DSM 432^T, *Blastobacter denitrificans* IFAM 1005^T, *Angulomicrobium tetradrale* Z-2821^T (= VKM B-1335^T), *Ancylobacter aquaticus* ATCC 25396^T, *Ralstonia eutropha* H16 DSM 428 and *Methylopila capsulata* VKM B-1606^T. 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^T was grown on minimal medium K, which contained (g l⁻¹): KH₂PO₄, 2.0; (NH₄)₂SO₄, 2.0; MgSO₄ · 7H₂O, 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 1 l medium was supplemented with 1 ml trace element solution (Doronina *et al.*, 1995). Methanol or methylamine was added to a concentration 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⁻¹), 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 between 0.5 and 3.0 g l⁻¹. 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 C₁ 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 (NH₄)₂SO₄ was replaced by 0.5% (w/v) KNO₃, after 1, 3 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, 3 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% H₂O₂ 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 methane 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 H₂/O₂/CO₂ (7:2:1, by vol.). Utilization of chloromethane was tested as described for DCM (Doronina *et al.*, 1995). To test alternative nitrogen sources, (NH₄)₂SO₄ 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) OsO_4 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 (T_m) 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'-AGAGTTTGATC-CTGGCTCAG-3' (8–27 by *E. coli* numbering); reverse primer, 5'-TACGGTTACCTTGTTACGACTT-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 Tet-z polymerase (BioMaster) and reaction buffer (100 mM Tris/HCl, pH 8.3, 500 mM KCl, 2 mM MgCl_2). 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 DM10^T was manually aligned to representative proteobacterial 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 100 nt) were computed by use of the correction of Jukes & Cantor (1969). The unrooted phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with bootstrap analysis of 100 trees and by using the programs of the TREECON package (Van de Peer & De Wachter, 1994).

RESULTS

Morphology

Strain DM10^T 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

Fig. 1. Electron micrographs of cells of strain DM10^T. Upper micrograph, negatively stained preparation; lower micrograph, ultrathin section showing cell wall structure. Bars, 1 µm.

singly or in pairs and sometimes in clusters. Reproduction was by binary fission. Special subcellular structures related to the autotrophic or methylobacterial 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 DM10^T 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 DM10^T grew well on mineral salt liquid medium with a gas mixture of $\text{CO}_2/\text{H}_2/\text{O}_2$, DCM (optimal concentration 0.05–0.1%, v/v), methanol (0.5%, v/v), methylamine (0.3%, w/v) or formate (0.05%, w/v) as the carbon and energy sources. Growth on C_1 substrates was stimulated by addition of yeast autolysate (0.01%) or thiamin/biotin (both at 20 µg l⁻¹). Growth was also supported by glucose, fructose, arabinose,

Table 1. Cellular fatty acid composition of strain DM10^T grown on PYG agar (48 h)

Fatty acid	Percentage of total fatty acids
Iso 15:0	0.3
Anteiso 15:0	1.5
Iso 16:0	0.7
16:1	1.5
16:0	19.7
Anteiso 17:0	1.0
Unsaturated 18	11.6
<i>c</i> 18:1 ω 7	57.6
18:0	6.0

maltose, mannose, sorbose, ribose, sucrose, sorbitol, mannitol, glycerol, inositol, dulcitol, adonitol, ethanol, acetate, 2-oxoglutarate, fumarate, pyruvate, succinate, malate, propionate, acetamide, L-glutamate or L-alanine. A gas mixture of CH₄/CO₂/O₂ as well as formaldehyde, di- and trimethylamine, chloromethane, thiocyanate, thiosulfate, dimethyl acetamide

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 (*c*18:1 ω 7) and palmitic (16:0) acids. Analysis of the cellular phospho-

Table 2. Enzyme activities in cell extracts of the strain DM10^T grown with DCM or methylamine

No activity of any of the following enzymes was observed (cofactors in parentheses): formaldehyde dehydrogenase (PMS), formate dehydrogenase (NAD⁺), hydroxypyruvate reductase (NADH), serine-glyoxylate aminotransferase [NAD(P)H], malate lyase (ATP, CoA), 3-hexulose-phosphate synthase, 2-keto-3-deoxy-6-phosphogluconate aldolase and glutamate dehydrogenase (NADH).

Enzyme (co-substrates)	Enzyme activity [nmol min ⁻¹ (mg protein) ⁻¹]	
	DCM-grown cells	Methylamine-grown cells
DCM dehalogenase (GSH)	118	0
Methylamine dehydrogenase (PMS)	0	120
Formaldehyde dehydrogenase (NAD ⁺ , GSH)	700	620
Formate dehydrogenase (PMS)	50	20
Hydroxypyruvate reductase (NADPH)	120	116
Phosphoribulokinase	70	92
Ribulose-1,5-bisphosphate carboxylase	120	145
Glucose-6-phosphate dehydrogenase (NAD ⁺)	60	67
Glucose-6-phosphate dehydrogenase (NADP ⁺)	60	70
6-Phosphogluconate dehydrogenase (NAD ⁺)	64	69
6-Phosphogluconate dehydrogenase (NADP ⁺)	64	65
Fructose-1,6-bisphosphate aldolase	30	44
Pyruvate dehydrogenase (NAD ⁺)	17	21
Citrate synthase	27	35
Isocitrate dehydrogenase (NAD ⁺)	22	18
Isocitrate dehydrogenase (NADP ⁺)	362	380
α -Ketoglutarate dehydrogenase (NAD ⁺)	26	28
Isocitrate lyase	12	15
Malate synthase	16	17
Glutamate dehydrogenase (NADPH)	75	81
Glutamate synthase (NADPH)	21	24
Glutamine synthetase (ADP, Mn ²⁺)	30	37

Fig. 2. Unrooted phylogenetic tree showing relationships between *Albibacter methylovorans* DM10^T and other members of the α -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.

lipids revealed the presence of phosphatidyl ethanolamine, phosphatidyl glycerol, cardiolipin, phosphatidyl choline, phosphoserine, lysolecithin and a minor unidentified phospholipid. The major ubiquinone is Q-10.

Metabolic characteristics

In order to establish the routes of primary and intermediary C₁ metabolism in strain DM10^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 CO₂ 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 C₁ assimilation was operative, due to the absence of the appropriate specific enzymes: hydroxypyruvate reductase (NADH), serine-glyoxylate aminotransferase, malate lyase and hexulose-phosphate synthase. Strain

DM10^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^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^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^T showed low levels of DNA–DNA relatedness (2–7%) to the following strains: *P. denitrificans* ATCC 17441^T, *P. methylutens* VKM B-2164^T, *P. aminovorans* JCM 7686^T, *P. aminophilus* JCM 7686^T, *Xanthobacter autotrophicus* DSM 432^T, *Blastobacter denitrificans* IFAM 1005^T, *Blastobacter capsulatus* IFAM 1004^T, *Angulo-*

microbium tetradrale Z-2821^T, *Ancylobacter aquaticus* ATCC 25396^T, *Ralstonia eutropha* DSM 428 and *Methylopila capsulata* VKM B-1606^T.

Comparative 16S rDNA sequence analysis

A total of 1409 nt of the 16S rDNA sequence was determined for strain DM10^T, 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 methylobacteria belonging to the genera *Paracoccus*, *Xanthobacter*, *Blastobacter* and *Ancylobacter*. Surprisingly, the matrix of full sequence similarity values for strain DM10^T and representative members of the *Proteobacteria* indicated a rather high identity (95.4%) to a recently described member of the α -2 subgroup, *Methylopila 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 DM10^T formed a single cluster with the type strain of the serine-pathway methylotroph *Methylopila capsulata* (bootstrap value of 100%). However, the scale of evolutionary changes in the 16S rRNA gene of strain DM10^T 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 DM10^T, argue strongly against its formal generic clustering with *Methylopila capsulata*.

DISCUSSION

Six generic names have been validly published for Gram-negative RuBP-pathway methylobacteria: *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 DM10^T and species of the genera *Xanthobacter* and *Blastobacter*. Although strain DM10^T resembled *Paracoccus* species on the basis of morphology, ubiquinone system, pathway of C₁ 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 *Methylopila capsulata*, strain DM10^T 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 DM10^T 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 methylovorans* 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. Chemolithoheterotrophic 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 (c18:1 ω 7) and palmitic (16:0) acids. Levels of DNA–DNA hybridization with representatives of the genera *Paracoccus*, *Xanthobacter*, *Blastobacter*, *Angulomicrobium*, *Ralstonia* and *Methylopila* 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 methylovorans*.

Description of *Albibacter methylovorans* sp. nov.

Albibacter methylovorans (me.thy.lo.vor'ans. M.L. n. *methylum* the methyl group; L. part adj. *vorans* devouring, digesting; *methylovorans* 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-xylose, sorbose, ribose, maltose, sucrose, D-sorbitol, D-mannitol, inositol, glycerol, dulcitol, adonitol, ethanol, acetate, 2-oxoglutarate, 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, novobiocin, nalidixic acid, tetracycline and lincomycin. The DNA G+C content is 66.7 mol% (*T_m* method). The type strain, DM10^T (= VKM B-2236^T = DSM 13819^T), was isolated in Switzerland from ground water contaminated with DCM.

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